THE MORPHOLOGY OF THE THIRD CHROMOSOME IN THE SALIVARY GLAND OF DROSOPHILA MELANOGASTER AND A NEW CYTOLOGICAL MAP OF THIS ELEMENT

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Received July 31, 1934

The present article is the second of a series of three papers dealing with the morphology of the chromosomes of *Drosophila melanogaster* as these appear in the salivary glands of larvae, and to the presentation of a new type of chromosome map. As in my paper on the X chromosome, which appeared recently in this journal, the brief descriptions and the numerous figures have been presented primarily with the view of assisting other investigators in the ready identification of the various landmarks along the two arms of the third chromosome, because it is clear that study of salivary gland material is going to play an important part in the future genetic studies on Drosophila. The cytological map we are able to present now is little more than a skeleton, in spite of the fact that some 33 translocations have been analyzed. The reason for this is two-fold. In the first place, a great many of the breaks are bunched between rather narrow genetic limits. Thus out of a total of 36, 17 fall between the genetic limits of scarlet and pink. In the second place, the markers used in the original experiment were rather widely scattered and neither the time nor, in some instances, have the necessary stocks been immediately available for the finer analyses needed to determine more exactly the genetic limits of these breaks. Miss META SUCHE is making these determinations and by the time this article goes to the printer we should be able to add new data.[1] In the meantime, the morphological positions of the breaks will be indicated. No doubt many other investigators have breaks in the third chromosome, the genetic limits of which have been relatively exactly determined, and it is believed that they will have no difficulty in placing these cytologically with the aid of the topographical maps presented in this study. In this way within a few years we should have a large number of gene loci placed within narrow morphological limits.

Aside from the usefulness of the topographical maps for the study of all types of chromosome rearrangements and the problems associated with this branch of cytogenetics, perhaps the points of greatest general interest center around the question of chromosome structure. For the present study shows very clearly that the third chromosome is differentiated

[1] I have substituted for the original figures 1 and 29 new chromosome maps presenting information concerning the third chromosome as of February 14, 1935. In most instances, the position of the gene loci indicated may be taken as approximately correct and is based on additional evidence not considered in this paper.

linearly into two types of areas, which are quite different morphologically. The greater part of both arms shows what we shall term, for the moment, a banded form. This corresponds, apparently, to the "euchromatic" area of HEITZ (1932) and probably carries most, if not all, of the active genetic material. In the region of the spindle fiber, however, there is a considerable amount of material which does not show a banded organization. In oogonial metaphase chromosomes this represents not only the primary constriction, or achromatic bridge, and the spindle fiber attachment zone but an appreciable amount of chromatin lying to either side of the achromatic bridge. This probably consists of inert chromatin, similar to that which the X chromosome carries, and which shows heteropycnosis (HEITZ) at the interphase in somatic mitoses. In the salivary gland, this area lies within, or better, is a part of, the "chromocenter" (HEITZ), or what I termed chromatic coagulum in my first papers. This inert chromatin HEITZ has termed "heterochromatin," a name which will be adopted here.

The translocations described were obtained by my colleagues and associates Dr. PATTERSON and WILSON STONE and the Misses SARAH BEDICHEK and META SUCHE, from a series of rayings carried out in the spring of 1933. A brief summary of their findings has recently appeared (PATTERSON et al 1934). The genetic analyses of the third to fourth translocation have been carried out by Miss SUCHE. At this place I wish to express to my colleagues my obligation and appreciation for the use of this material.

Two points concerning the behavior of the chromosomes in salivary glands must be borne in mind. The first is that in old larvae homologous chromosomes undergo somatic synapsis. The drawings of normal chromosomes in practically all cases are made from paired elements, the pattern of the segments being essentially the same before and after synapsis. The second point is that the two arms of the third chromosome appear as separate elements, the connection between the two being masked by the chromocenter to which they are attached along with the other elements.

In earlier papers (PAINTER 1934a, 1934b) the aceto-carmine technique has been described in sufficient detail. Here we will confine our remarks to some of the factors which alter the images we get in well-stained preparations.

STAINING

One of the most vexing details is the preparation of good aceto-carmine stain. One may prepare two batches of this fixative stain, in seemingly just the same way, and find that one works much better than the other, for some unknown reason. Granted good aceto-carmine, the following points may be noted: (1) there is some variation in the degree the stain will take in different sets of larvae; (2) in lightly stained preparations we

get a greater contrast in the patterns of lines and bands but many of the finer details will not be easily visible; (3) in old slides, or deeply stained preparations, the lines and bands of the faintly staining "achromatic" areas become much more prominent and, in general, there is much less contrast.

DISTORTIONS

These are due, presumably, to the mechanical effects of crushing nuclei in order to separate the chromosomes. It must be remembered that all elements are anchored to the chromocenter which lies on one side of the nucleus (PAINTER 1933a) and when the nuclear wall is broken, this connection is usually retained. The length of time of fixation prior to crushing may enter here. Distortions express themselves in a number of ways: (1) the breaking of chromosomes or arms, (2) twisting and bending at sharp angles, (3) local stretching when stress is thrown on a short area, an effect most often seen in the segments close to the chromocenter, (4) mashing and (5) telescoping or crowding together of adjoining segments. This last is always to be reckoned with because it results in the close apposition of a number of bands forming broad deeply staining areas, not usually seen. In general, areas with heavy chromatic bands are less subject to distortion than relatively achromatic segments.

Throughout this series of papers we speak of the chromosomes as being made up of segments, but a word of explanation should be inserted here. The shallow constrictions which bound the segments are sometimes very conspicuous and constant but in other areas they may be more variable in expression. At the outset the investigator will do well to pay more attention to the pattern of lines and bands.

In making the topographical charts of the left and right arms the same procedure has been followed as in my study of the X chromosome. Each segment is based on a camera lucida drawing of some chromosome, the whole chart being made by putting these typical segments together. The main emphasis has been placed on the pattern of the lines or bands, as these appear in a moderately stained preparation, together with the typical length and breadth of the segment, as I have observed it. In a study involving as much detail as the present one, the author can not hope to have escaped making some errors in the emphasis placed on the lines of some segments and it is to be expected that as limited areas are subject to intensive study the typical pattern may be altered somewhat, especially with regard to the finer details. However, since no segments on the type chart have been inked in without viewing at the same time a good preparation of the area involved under the microscope, the reader may be sure that something was seen at the point indicated even though its relative size or degree of staining may be incorrectly represented.

In the study on the X chromosome individual segments were designated by the names of the genes they were thought to contain. In the case of the third chromosome this information is so fragmentary that it has seemed desirable to adopt a temporary designation for landmarks. The genetic crossover map is represented above the topographic map and it is convenient to refer the various landmarks to the scale of the former. It must be understood that we do not mean to imply that a segment which lies under 70, for example, on the crossover scale necessarily carries the gene for sooty.

The point of spindle fiber attachment has been commonly assumed to be close to 48 crossover units from the left-hand end. So on the crossover scale the left arm shows 48 units and the right 57. We have thus disregarded the size of the spindle fiber region and the inert material to either side of it although in the oogonial metaphase this represents possibly 1/8 to 1/10 of the total volume of the entire chromosome. The crossover value of various genes has been taken from the Drosophila Information Service Number 1, March, 1934.

THE NORMAL TOPOGRAPHY OF THE LEFT ARM

Figure 1 shows the typical morphology of the left arm of the third chromosome. Figure 2 is a camera lucida drawing of this arm as it appeared in one of my preparations. With the aid of the numbers (from the crossover scale) placed along the latter, the reader will have no difficulty in identifying the various segments.

The most easily identified landmark of this arm is the right-hand terminal segment lying at the right in figure 1. This, the spindle fiber end, is very blunt and is usually more or less covered by a part of the deeply staining granules of the chromocenter to which this arm is anchored. A detailed description of this latter region will be given later in this paper. At the right-hand tip there are three heavily stained bands, one or more of which may be concealed by the chromocenter. To the left (roughly below 46 on the crossover scale) is a clear area traversed by faintly staining bands. It is this clear area with its faint bands lying between heavily stained sectors which usually catches the eye. Next comes a set of three broad deeply staining bands, the two outside ones being especially heavy while the middle one is lighter. Then comes a double and then a narrower band followed by two faintly staining bands. The segment is bounded at the left (below 44) by three narrow but sharply staining bands. A comparison of the chart with the terminal segments of figures 2 to 5, will show these features, as well as a number of the translocation figures (for example, figures 15, 21, 25, 26 and 28).

The next outstanding feature is a series of relatively achromatic seg-

ments lying in the region below about 30 to 38 on the crossover scale. Beginning with the narrow collar below 38, in figure 1, we have two more or less swollen segments bounded at the left by three heavy bands. A narrow heavily staining line separates these two segments which individually show very faintly staining lines. These two segments show a striking variation in diameter, though within an individual I am under the impression that the form is fairly constant. In some larvae they may be as much as three times the average diameter of the element, when, of course, they are very conspicuous, in other larvae they do not exceed the normal diameter by very much. Figures 1 and 2 show the usual size, in 3 and 5 the segments are relatively narrow while in figures 6a to c we have examples of enlarged segments. When I first began to study the salivary glands of larvae, enlarged segments were the rule but within recent months I have encountered fewer cases.

The next three segments are relatively achromatic and while not so variable as to width, seem to be structurally weak for the arm bends here more frequently, perhaps, than elsewhere and telescoping of segments is rather frequent.

The next outstanding landmark is a swollen segment lying at about 21 under the crossover scale. This is, ordinarily, the second most striking feature of this arm. Across the point of greatest diameter is a band of faintly staining lines. Figures 2 and 7 show this feature in typical form.

Lying to the left of 21, we have a series of segments, part of which showing heavily stained bands and others are more or less achromatic. None of these is outstanding nor easily picked up, and breaks in this region have to be carefully identified by beginning either at the left-hand end or else with the enlargement at 21. The left-hand end itself is not especially easily recognized until one becomes quite familiar with it. Figures 2, 8, and 9 show characteristic drawings. The bands of the terminal bulb stain lightly. This segment is constricted, at the right by two narrow but deeply staining bands, then come some rather broad diffuse bands followed by a heavily stained line. This description may be concluded with the remark that often the lines at the left end, which I have represented as diffuse and rather lightly staining, may be quite prominent in heavily stained slides.

THE NORMAL TOPOGRAPHY OF THE RIGHT ARM

This arm is the longest element in the nucleus and one of the easiest to identify by either the spindle fiber or the right-hand free end. In spite of this, it has proved to be the most difficult of all the elements for which to present a typical morphological map, due principally to the great length and the attendant distortions especially in the mid reaches. Added

EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida at table level. For salivary gland chromosomes a magnification of 900 diameters at the eye piece was used. Oogonial chromosomes were drawn either with a magnification of 2400 or 1800 diameters at the eye piece. The drawings have been reduced about a third in the plates. Throughout this paper I have used our local terminology for the III to IV translocations. Recently my colleagues have adopted a new set of symbols. Translocation VI 1, for example, is now written TA 3–4, 1 which means, a translocation found at Austin, Texas involving the third and fourth chromosome, No. 1. In similar manner VI 36 would be written TA 3–4, 36, and so on.

FIGURE 1.—This is a cytological map showing the typical morphology of the euchromatic portion of the left arm of the third chromosome. The scale above the figure is a crossover map. Below the figure the numbers refer to the various translocations studied. Lines shown from the crossover scale to the map show the limits of various gene loci.

FIGURE 2.—A drawing of the euchromatic portion of a left arm which was intact. The numbers placed along the drawing will assist the reader in identifying the various landmarks on the cytological map (figure 1).

FIGURES 3 to 5.—Presented to show the topography of the spindle fiber end of the euchromatic area.

FIGURES 6a to 6c.—Presented to show variations in the size of the achromatic enlargements lying between about 34 and 37 under the crossover scale.

FIGURE 7.—This is a figure of the spindle-like enlargement lying under 21 on the crossover scale.

FIGURES 8 and 9.—These are drawings presented to show the topography of the left-hand end of the left arm.

FIGURE 10.—This is a drawing of the left-hand end of the left arm of a larva heterozygous for translocation VI 34. The normal end of the synapsed homologs is shown to the left and below while the fourth chromosome, which has replaced the normal tip of one element, is shown above.

FIGURE 11.—This figure is from a larva heterozygous for the VI 22 translocation. The normal component lies to the left while the element carrying the tip of the fourth chromosome is shown to the right.

FIGURE 12.—This drawing was taken from a larva hyperploid for the VI 52 translocation, and shows the III L–IV component, not all details drawn.

FIGURE 13.—This is a drawing taken from a larva heterozygous for translocation VI 23.

FIGURE 14.—From a larva heterozygous for translocation IV 13.

FIGURE 15.—From a larva heterozygous for translocation VI 12. The aberrant element with its tip from a fourth chromosome lies below synapsed with the normal homolog above. The dotted lines to the right in the figure shows where another element crossed.

FIGURE 16.—From a larva heterozygous for translocation VI 8. The aberrant III L–IV element which received its spindle fiber attachment from a fourth chromosome is shown above to the left. The tip of the fourth which covers the broken end of the left arm lies to the right, and is somewhat mashed out.

FIGURES 17 and 18.—From individuals heterozygous for translocation VI 56. Figure 17 shows the salivary gland chromosomes while figure 18 is from a dividing oogonium.

FIGURES 19 and 20.—Showing two drawings taken from larvae heterozygous for translocation VI 14.

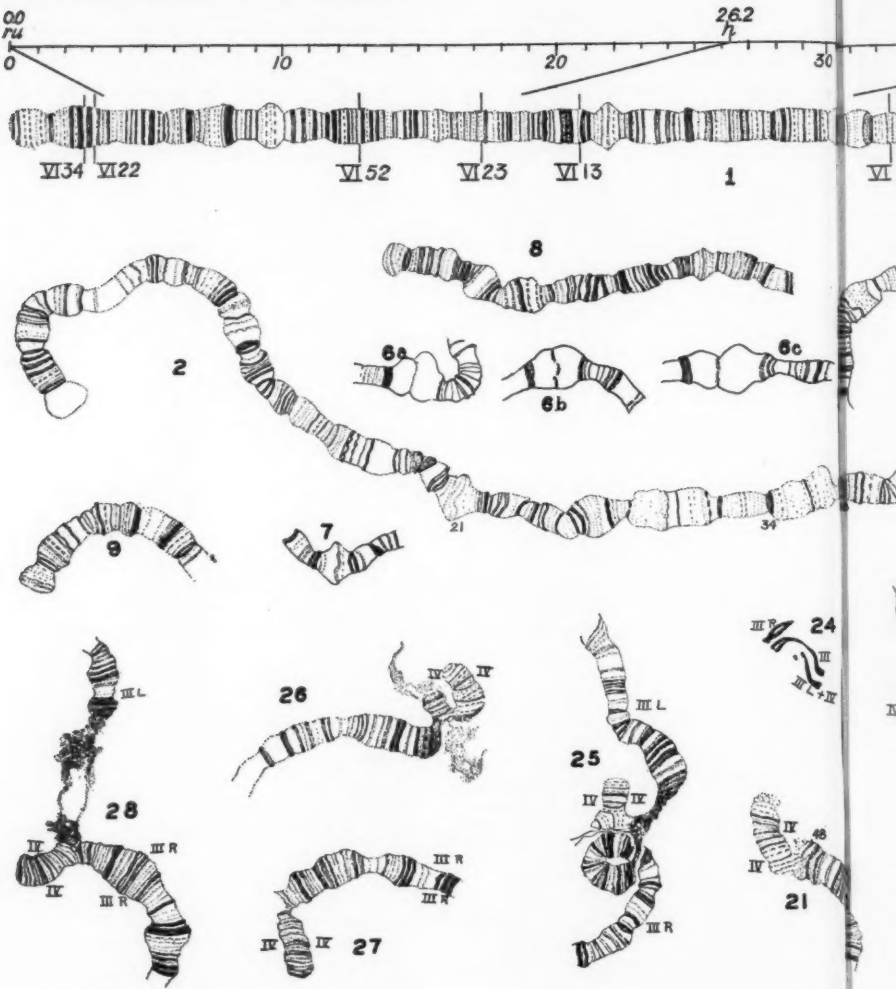
FIGURES 21 to 23.—From individuals heterozygous for translocation VI 36. Figure 21 is from the salivary gland, and figures 22 and 23 show the third chromosomes in dividing oogonium.

FIGURES 24 and 25.—From individuals heterozygous for the VI 45 translocation. The condition of the third chromosome, as these appear in dividing oogonia, is shown in figure 24. Figure 25 was taken from the salivary gland.

FIGURE 26.—From a larva heterozygous for translocation VI 31.

FIGURE 27.—From a larva heterozygous for translocation VI 4.

FIGURE 28.—From a larva heterozygous for translocation VI 27.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factors are a tendency of certain regions to telescope or stretch and a marked similarity of the patterns of several segments which lie close together.

We will begin with the spindle fiber end which lies to the left in figure 29 (plate 2). A camera lucida drawing of an entire arm is shown in figure 30. The numbers placed along this figure refer to the crossover scale of figure 29.

The right arm tapers rather sharply at the end which is attached to the chromocenter and then broadens out into a series of three or four ill-defined and relatively achromatic segments. Then it narrows again at a point below about 52, on the crossover scale. This club-like achromatic end is one of the outstanding morphological features of this arm and since it often pulls free from the chromocenter, it is frequently seen. In some preparations the paired and synapsed homologs show a bifurcated tip (figures 31–33); at other times there are two ring-like vesicles at the end, as in figure 30. Where the latter were first observed, I was struck with their similarity to the "insertionslücke" which BĚLAR (1929) figured for Chorthippus and I was inclined to interpret them as the points of spindle fiber insertion. As it turns out, however, these structures are simply the stumps of the protoplasmic strands (viewed head on) which run into the chromocenter and serve, directly or indirectly, to connect the two arms together (see p. 315).

The next outstanding feature is an enlarged spindle-shaped segment lying between 62 and 63. This is a very useful landmark and various drawings of it are shown in figures 30, 31, 34 and 40. From this segment on to about 71 we have a series of heavy bands bounding more or less achromatic segments which seem to be structurally weak as they often stretch a good deal and thus make the appearance of this area quite variable. Here the investigator should follow the pattern of the bands very closely. Beginning about 71 we have two segments which are also apt to stretch or telescope and often show little sign of the constriction figured for the typical arm. These are followed to the right (75–77) by a narrow area which leads up to a segment with a very striking pattern. The features which catch the eyes first are the heavy bands lying below 77 and a trifle past 79. This segment tapers to a constriction just below 80 on the scale.

The next two segments are also very characteristic, the sequence of the heavy bands of the segment lying below 82–83 being outstanding. Note the sequence of two broad, one narrow and then one broad heavily stained band. Sometimes, as in figure 30, these bands appear to bound short achromatic segments.

Beginning between 87 and 88 we again have a section showing two

broad, one narrow and one broad band, and care must be taken not to confuse this area with that lying below 83. Between these heavily banded segments is a relatively achromatic section which, for some reason, seems to be little subject to distortion, and its pattern can generally be observed.

Below 90 to 94 is a lightly stained area which is very useful as a landmark because the narrow bands are in series of three. From 94 to the right we have a number of ill-defined segments with many heavy bands. The details of the pattern are shown in figure 29. Note especially the pattern on either side of the VI 30 break. To the left of this break we have three thin but sharply staining lines, and to the right four conspicuous bands with the sequence of one broad, one narrow and two heavy bands.

The outstanding landmark of the free end of the right arm is between 103 and 104 and consists of four conspicuous bands in the order, beginning at the left, of one broad, one narrow and then two broad lines.

TRANSLOCATIONS

Four general types of translocations have been found during the course of this study. (a) In the majority of cases we have mutual exchanges between the euchromatic or banded areas of one or the other arm of the

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FIGURE 29.—A cytological map of the euchromatic area of the right arm of the third chromosome showing the typical morphology as this appears in salivary glands. The crossover scale is above and the translocations studied are indicated below the figure.

FIGURE 30.—A drawing of the euchromatic area of a right arm which was intact.

FIGURES 31 to 33.—Three drawings of the spindle fiber end of the right arm.

FIGURE 34.—Presented to show, in more detail, the character of the enlargement which lies under about 62–63 of the scale of figure 29.

FIGURES 35 to 37.—These are drawings to show the morphology of the right-hand tip of the right arm.

FIGURE 38.—From a larva heterozygous for translocation VI 37.

FIGURE 39.—This drawing is from a larva heterozygous for translocation VI 9, with a few details omitted. The fourth chromosomes are mashed out and appear unduly broad.

FIGURE 40.—From a larva heterozygous for translocation VI 1. This figure is discussed in detail in the body of this paper (see p. 309).

FIGURE 41.—From a larva heterozygous for translocation VI 20.

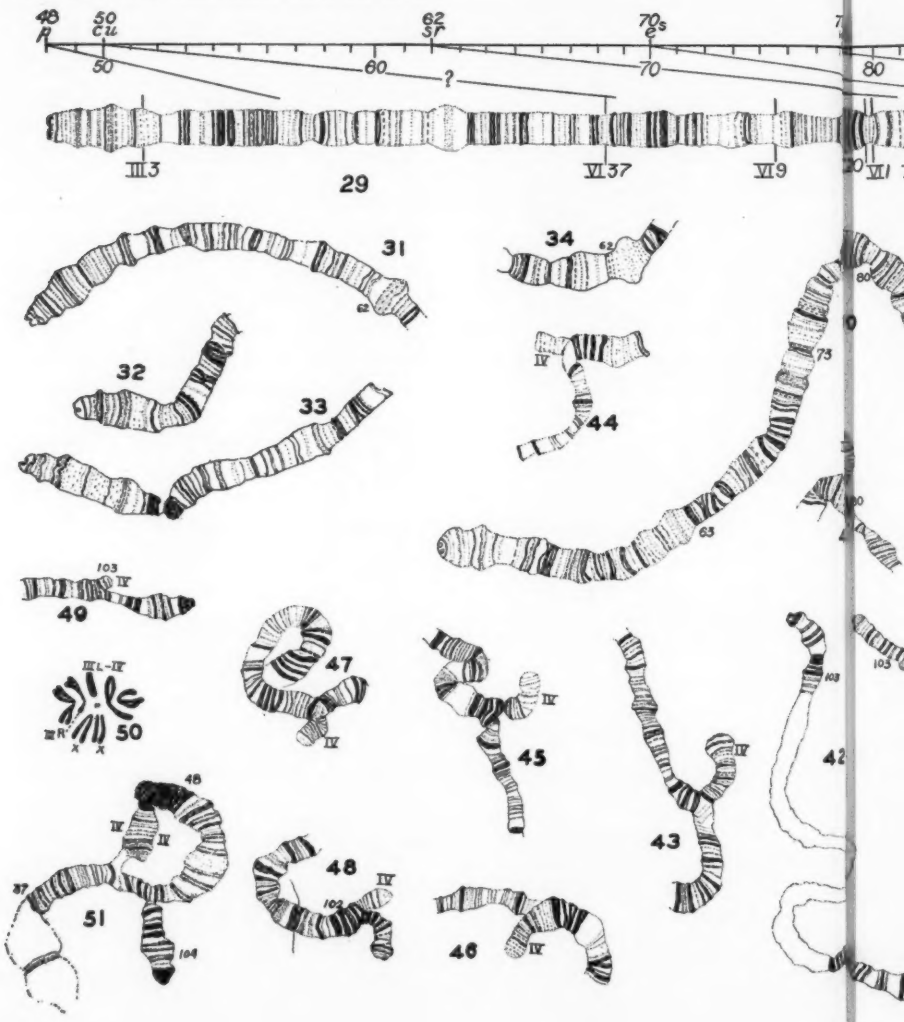
FIGURE 42.—This drawing was taken from a larva hyperploid for translocation VI 43. Most details have been omitted from the III R–IV segment.

FIGURES 43 to 46.—Various breaks in the right arm taken from larva heterozygous for the translocation. Figure 43 is from translocation VI 60, figure 44 is from VI 28, figure 45 is from VI 2 and figure 46 is from VI 39.

FIGURE 47.—From a larva heterozygous for translocation VI 30.

FIGURE 48.—From a larva heterozygous for translocation VI 24.

FIGURES 49 to 51.—These figures are taken from larvae heterozygous for translocation VI 44. Figure 49 shows the condition of the right end of the right arm. Figure 50 is an oogonial metaphase, while figure 51 shows the normal and aberrant left arms. The case is described in detail on p. 314.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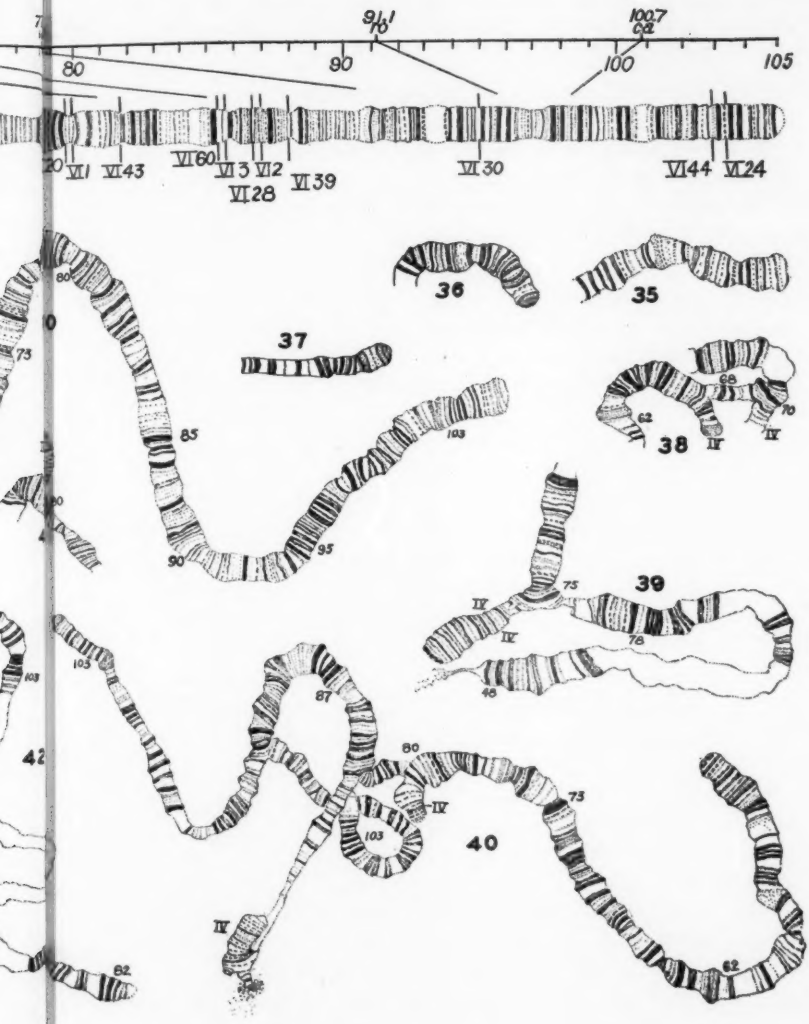
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third chromosome and a fourth chromosome. As a result the distal (to the spindle fiber) part of the arm receives its spindle fiber attachment from the fourth with more or less of the euchromatin of the fourth, and the proximal part of the arm has its stump covered by the free end of the fourth. In oogonial metaphase plates, the distal part of the arm with its spindle fiber from the fourth appears as a rod-shaped chromosome. Such cases were studied by the salivary gland method in larvae heterozygous for the aberration. (b) There are a number of cases, however, in which one or the other arm of the third chromosome is replaced apparently by an intact fourth chromosome forming a j-shaped element. That is to say, in some way the spindle fiber end of the fourth becomes attached to the spindle fiber end of one arm, while the displaced arm acts as a free chromosome. In such cases the salivary glands show, as a rule, the normal configuration of all elements being attached separately to the chromocenter, but in oogonial metaphases the j-shaped compound chromosome and the displaced rod-like arm are readily identified. For this reason, most of the translocations falling between the loci of scarlet and pink were first studied in oogonia of female flies heterozygous for the exchange. (c) A few cases have been encountered in which a piece of one arm of the third becomes attached to the spindle fiber end of a fourth which is, so far as I can see, morphologically intact. In such cases, in salivary glands, we find the piece of the arm attached apparently to the fourth chromosome. (d) There is one case in which the tip of the right arm became translocated to the stump of the left arm, the distal end of the latter being translocated to a fourth chromosome. Such cases are easily analyzed by the salivary gland method but in oogonial divisions are unintelligible.

Due to somatic synapsis, in heterozygous larvae homologous parts tend to unite, just as they do at meiosis, and by comparing the patterns of the normal and the aberrant elements, one can tell very exactly just where the latter is broken. As I shall discuss in detail elsewhere, somatic synapsis seems to be a progressive process, and in any given cell it may not have been entirely completed. Figure 40 of plate 2 is presented to show the nature of the material with which we are dealing. This represents the right arm of the third chromosome in a larva heterozygous for a mutual exchange with a fourth chromosome, the break falling between the loci for the genes curled and stripe. This is the VI 1 break shown near the middle of the right arm in figure 29. Beginning at the spindle fiber end, which lies to the right in figure 40, the reader will have no difficulty in identifying the outstanding landmarks of this arm. The normal and the aberrant elements, beginning at the spindle fiber end of the euchromatic area, have united completely up through the conspicuous segment which lies at about 80. Here the two diverge and the lower element ends, showing

the pattern of about three-fourths of the fourth chromosome. The break in the arm originally was obviously just to the right of the two very heavy bands which lie close to 80 in figure 29. The normal and the spindle fiber end of the broken fourth chromosomes show in the lower part of figure 40 to the left. Here it will be noted that the two are synapsed for about a fourth of the length of the normal fourth (which lies on the left) and then the element at the right joins the normal right arm with which it synapses for some distance. At about 90 on the chromosome map (figure 29) the two elements diverge again, that is, they failed to synapse although the parts are homologous.

This is a fair illustration of the type of preparation from which the position of breaks have been determined in the following sections. In most of the other translocation figures only short sectors of the normal and aberrant arms have been drawn. In a few cases, as noted, the III L–IV component (or III R–IV) is shown by itself.

Translocations in the euchromatic area of the left arm

The topographical chromosome map (figure 1) shows that eleven breaks, accompanied by translocations, have occurred in the banded or euchromatic portion of the left arm. These will be considered in turn, beginning at the extreme left.

VI 34 and VI 22. Both of these translocations broke the left arm in the subterminal segment, as figure 1 shows. Figure 10 is from a larva heterozygous for VI 34 and may be of interest because it shows that all of the banded area of the fourth chromosome is attached to the proximal portion of the left arm. That is to say, the exchange between the fourth and the left arm broke the fourth beyond the banded portion of the latter but still distal to its spindle fiber attachment zone. Such cases have been frequently observed. In figure 11, showing the VI 22 translocation, the normal element lies to the left while the aberrant arm with its cap of fourth chromosome material is on the right. The two elements are synapsed below the point of breakage. Genetically both the VI 22 and VI 34 are to the left of roughoid (0.0) so that this gene must lie to the right of the points of breakage, as figure 1 shows.

VI 52. Figure 12 was taken from a larva which was hyperploid for this aberration, the duplicated piece being the left-hand tip with its spindle attachment from the fourth chromosome. The left arm is broken at about 12, as shown by figure 1. Genetically the left arm is broken between roughoid (0.0) and hairy (26.2) and thus the locus for roughoid must lie somewhere between the VI 22 and the VI 52 break.

VI 23. Figure 13 shows the normal homolog above and running to the left, while the aberrant element is drawn out to the right because its

fourth component has synapsed with the normal fourth. Here again it is to be noted that the fourth chromosome was broken between the euchromatic portion and its area of spindle fiber attachment. Genetically, the breeding data indicate that this break is very close to hairy so we can place the locus of this gene close to the line marking the VI 23 break in figure 1.

VI 13. Figure 14 shows that the left arm was broken close to 20 on the crossover scale of figure 1. Genetically, this break is between the gene loci of hairy (26.2) and thread (42.2); thus hairy must lie to the left and thread to the right of it.

VI 12. Figure 15 shows that the left arm is broken just to the left of the three heavy bands shown between 33 and 34 of figure 1. Genetically this break is between thread (42.2) and scarlet (44).

VI 8. This translocation also falls between the genetic limits of scarlet and pink. The point at which the left arm was broken is shown by figures 16 and 1.

VI 56. Genetically, the preliminary tests indicate that this break came between scarlet and pink. In salivary gland material, as figure 17 shows, the left arm is broken a trifle past 40 on the crossover scale of figure 1. The oogonial metaphase plates of females heterozygous for this translocation are of extreme interest. Figure 18 is almost a prophase stage taken from an attached-X female. The normal and broken third chromosomes are shown below and to the left in the figure. Note, that the III L-IV element is much shorter than the normal arm, and, in turn, that the broken third is much prolonged beyond the primary or spindle fiber constriction, in other words, the truncated left arm shows a length of about a fourth of the total length of the arm. If the genetic analysis proves to be correct, this means that the locus for scarlet must lie much farther away from the spindle fiber, in oogonial chromosomes, than its crossover value would indicate. Incidentally, the truncated left arm shows the secondary constriction described by DOBZHANSKY and others.

VI 44. This is a complicated case which will be described along with breaks in the right arm. On figure 1, the point at which the left arm was broken is shown between 41 and 42. The (limited) genetic data indicates that this break is between scarlet and pink.

Translocations VI 36 and VI 14 are of extreme interest. Genetically they broke the third chromosome between scarlet and pink, but morphologically the break comes right at the border line between the banded or euchromatic area of the left arm and the region of the chromocenter. A study of oogonial metaphases should show us, therefore, how much of a contribution to the left arm is made by chromocenter material. Figures 19 and 20 show the right-hand tip of the synapsed normal and aberrant

arms of VI 14. In figure 19, it will be observed that only two of the three heavy bands are shared in common, the normal component, which is below, shows three. This means that the left arm broke between the second and third band with which the euchromatic area terminates. Figure 20 shows the connection with the fourth chromosome at the end distal to the spindle fiber. Figure 21 shows, likewise, that in VI 36 the left arm is broken between the second and third of the heavy terminal bands and that one component of the synapsed arm is attached to the distal end of a fourth chromosome which, in turn, is synapsed with a normal fourth. Figures 22 and 23 are oogonial metaphase plates of flies heterozygous for the VI 36 translocation. It will be noted at once that there is a considerable amount of chromatin beyond the spindle fiber constriction of the broken third. This chromatin represents something like a sixth or an eighth of the total length of the left arm and must be derived almost wholly from the chromocenter region. Oogonial division of VI 14, which I have not figured, shows essentially the same conditions as VI 36.

Translocations occurring within the chromocenter region

The exchanges occurring in the region of the chromocenter are of two general types. In the first type, one arm of the third is simply replaced by a fourth chromosome. As far as I can determine, all elements are morphologically intact and the new association of one arm of the third with a fourth is at the spindle fiber ends of the two elements. As a result, in oogonia we find the broken third represented by a rod-shaped arm of normal length, and a j-shaped element, the short arm of the j being formed by the fourth chromosome. Two cases of this sort are illustrated by the figures, altogether seven cases of this sort have been studied.

VI 45. Figure 24 shows the condition of the third chromosomes in a female heterozygous for this exchange. The several elements are labelled. It will be noted that the fourth attached to one arm of the third (in this case the left) is the same size as the normal fourth. In salivary glands (figure 25) we find the paired right and left arms synapsed up to the point at which they enter the chromocenter. The two fourth chromosomes which lie between these ends are synapsed for a greater part of their length but they diverge at the spindle fiber ends, one of which enters the chromocenter close to where the left end is attached. Morphologically, the two fourths and the synapsed arms of the third are entirely intact. It is clear, therefore, that the association between the left arm and the fourth has been established within the chromocenter. Figure 26 shows the condition of the salivary gland in translocation VI 31. Here again we see the two synapsed left arms, which are intact connected by a strand to a mass of chromocenter material to which one of the two paired fourths

is attached. Oogonial plates of other translocations of this sort are shown in text figure B (p. 319).

There are two translocations occurring in the region of the chromocenter, for which the genetic data indicates that the break is close to pink, or is on the opposite side of the spindle fiber attachment zone from the cases previously considered. Figure 27 shows the salivary gland condition of larvae heterozygous for VI 4. Here it will be noted that the euchromatic area of one of the two (paired) right arms is attached to the spindle fiber end of one of the (paired) fourths. In oogonial plates of flies heterozygous for this translocation (see VI 4, text figure B) we find an unexpected situation. The figure is taken from a female of the attached-X stock. The broken third chromosome is represented by two j-shaped elements. One of these is obviously formed by the union of a normal-sized fourth with the right arm, while the left arm has a considerable amount of chromatin lying beyond the spindle fiber zone. This can only be interpreted in one way. The association between the right arm and the fourth is just at the edge of the euchromatic zone of the former, and the knob of chromatin attached to the left arm represents, simply, the contribution which the chromocenter makes to the right arm in metaphase plates. Translocation VI 27, as figure 28 shows, is another example of this same sort, and oogonial plates show essentially the same condition found for VI 4.

Translocation in the euchromatic area of the right arm

The genetic data from translocations VI 31 and VI 27 described above indicated that they broke the third to the left but relatively close to pink (48). The salivary gland material indicates that the exchange was on the edge of the euchromatic area of the right arm, hence the locus for pink must be farther to the right. The only other break close to the spindle fiber region of the right arm is one known as X-IV, III, 3. It is a complicated case involving an apparent fusion of the X and a fourth chromosome and the association of a short piece of the right arm of the third with them. This case is dealt with at length in a joint paper with Mr. WILSON STONE (now in press). The point which concerns us here is that the fragment of the right arm of the third broke off a trifle past 51 on figure 29. When this piece (from 48 to 51) was tested genetically by Mr. STONE, it was found not to cover pink, so the locus of the latter must lie to the right of 51 on figure 29.

VI 37. This is a complex case which has not been worked out in all respects, but the genetic evidence indicates that the right arm broke between pink (48) and curled (50). Figure 38, taken from a heterozygous larva, shows that the right arm is broken close to 68 on figure 29. The locus for pink therefore lies somewhere between 51 and 68, on the topographic map (figure 29) and curled must lie to the right of 68.

VI 9. Genetically this break falls between the loci of curled (50) and stripe (62). Morphologically as figure 39 shows the break is somewhat beyond 74 on figure 29. In this case (figure 39) the paired fourths are mashed out and hence appear unduly broad.

VI 20 and VI 1, both broke the right arm close to 80. Figure 40 is a drawing from VI 1, already described, and figure 41 is taken from VI 20. Both of these breaks are between curled and stripe.

VI 43. As this is written the genetic evidence indicates that the locus of stripe (62) is very close to this break. Morphologically (figure 42 taken from a female hyperploid for the III R-IV piece) the break occurred close to 81 on figure 29.

We now have five different translocations which are genetically all beyond sooty (70) which broke the right arm in nearly the same place (between 85 to 88+ on figure 29). VI 60 is shown in figure 43. VI 28 is shown in figure 44. VI 2 in figure 45 and VI 39 in figure 46. No figure is given for VI 3. The locus for sooty must lie to the left of about 85 on figure 29.

VI 30. This translocation falls genetically between the loci for white ocelli (77.2 and rough 91.1). Figure 47 shows that this arm is broken close to 95, on the topographical map.

VI 24. This translocation broke the right arm beyond the locus for claret (100.7). Figure 48 places the morphological position of this break a little beyond 103 on the topographic map (figure 29).

VI 44. This is a complex translocation which will be dealt with in some detail. When the case was first studied cytologically, the genetic evidence was limited to the fact that crossing over was reduced in the left arm. When the salivary gland nuclei were examined almost the first cell examined showed the condition given in figure 49. Obviously, the right arm is broken close to 103 on the topographic map. For the time being no further cytological analysis was made, but later oogonial divisions of heterozygous flies were examined and revealed the condition shown in figure 50. It appeared as if the right arm was broken close to the spindle fiber region and that the III R-IV piece was about three-fourths the length of the normal right arm. The salivary glands were again studied and figure 51 tells the story. Here we see attached to a segment of the left arm the portion of the right arm missing in figure 49. At the same time, the remaining portion of the left arm (distal to the break) is translocated to a fourth chromosome. This condition explains, of course, why crossing over is reduced in the left arm. Recent genetic tests by Miss SUCHE indicate that the break in the left arm is between scarlet and pink. This case is of great interest not merely because of its complications and the demonstrated value of the salivary gland method, but it should put us

on guard against hasty conclusions drawn from oogonial chromosome studies.

THE CHROMOCENTER REGION

In the nuclei of salivary gland cells there is an amorphous mass of chromatic material to which all chromosomes or arms are attached. This mass is termed a chromocenter by HEITZ (1929) and according to this author is formed by the fusion of the heteropycnotic chromatin carried by some of the chromosomes. HEITZ (1933) has shown for *D. melanogaster* that during the interphase of somatic mitoses the Y chromosome (when present), the proximal half of the X, and small sectors of the second and third chromosomes adjacent to the spindle fiber region, do not become diffuse following the telophase but remain condensed and deeply staining (heteropycnotic). It is this "heterochromatin" which unites to form a "sammelchromocentren" or chromocenter for short.

In aceto-carmine preparations the chromocenter appears to be made up of coarsely granular and fibrous material in which there are many apparent vacuolated spaces. It stains deeply and seems to be one continuous mass.

In any given nucleus the position occupied by the spindle fiber ends of the chromosomes or arms seems to be largely the chance result of how these happened to lie with relation to the plane of the cover glass when the nucleus was mashed out and for this reason contiguity is of little significance. There is, however, some evidence which points to a definite orientation of the chromosomes about the chromocenter. If the material of the chromocenter is more or less broken up, in mashing the nucleus, it often happens that the two arms of the third chromosome will be found attached to the same piece. More frequently, however, one or the other arms will be found attached to the same bit of substrat to which the fourth chromosomes are anchored. This condition is extremely common and suggests that the points where the two arms of the third are attached to the chromocenter is close to where the fourths normally are inserted. The fourths being relatively very short are less apt to be displaced than the longer elements.

When all the elements are anchored to the chromocenter there is no hint of any direct connection between the two arms of either the second or third chromosome. It is only when an arm is pulled away from the chromocenter that we have an opportunity to determine the nature of the connection. The image we get depends largely on the age of the preparation. In slides a day or two old the blunt end of the left arm almost invariably shows a good deal of the chromocenter material adhering to it (text figure A1) and this may be strung out for some distance. The connection must be a very intimate one, but one gains the impression that the chromocenter material is simply adhering to the outside of the tip,

just as would happen if a match stem were inserted into some sticky substance and then withdrawn (text figure A, also figures 15, 20, 25, 26 and many others). On the other hand, the connection between the right arm and the chromocenter must be a very loose one because it seems to pull free easily and usually none of the chromocenter material adheres to it.

If now, we examine a slide a few minutes after staining, in favorable cells where an element is pulled away a little from the chromocenter, we can observe arising from the ends of the paired homologs of all the elements, usually two thin and achromatic strands of protoplasm. After a few hours this connection usually dissolves or breaks, a fact which was

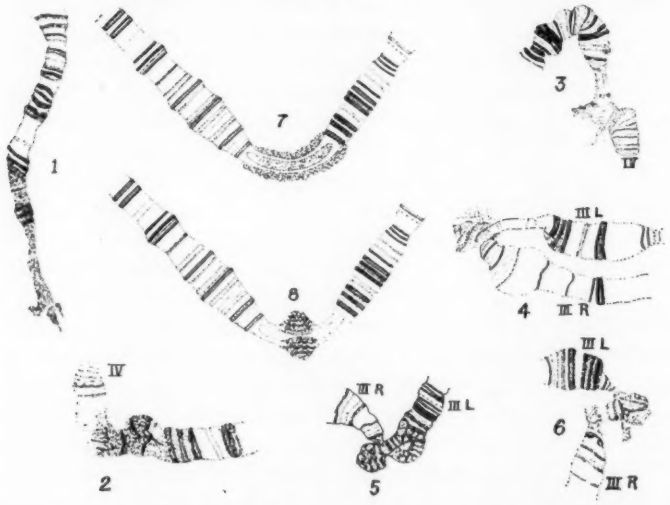

FIGURE A.—Figures A-1 and 2 show the close association between the euchromatic area of the left arm and the material of the chromocenter. Figure 3 shows the achromatic strands connecting the right arm to the chromocenter. Figures 4, 5 and 6 show how the left and right arms may be connected together. Figures 7 and 8 are diagrams to illustrate two possible ways of interpreting the nature of the connection between the two arms of the third chromosome.

accidentally discovered when an especially good illustration was left over night before drawing. Text figure A2 shows a typical case for the left arm and A3 a typical case for the right arm and it will be noted that the points of insertion are near the paired fourth chromosomes. In these and some of the following illustrations it is difficult to represent the achromatic character of these protoplasmic strands. They are usually so transparent as to show only with reduced lighting, but sometimes they show a faint suggestion of banding or they may be more or less covered by the granules of the chromocenter. In the latter event the granules seem to be adhering

to the outside of the strands. Text figure A4 is an interesting illustration. We see arising from a mound at the tip end of the left arm, a single transparent strand which runs to the base of the right arm, but which does not connect directly with it, so far as I can observe. At the end of the right arm (to the left) there is a mass of chromatic material which I interpret as the remains of the other strand which has broken (we are dealing with synapsed homologs) and some material from the chromocenter. Text figure A5 shows two processes, in this case apparently covered with chromatic granules, arising from the ends of the synapsed left arms and running to and connecting with the synapsed right arms. Text figure A6 shows the two arms connected to a common mass of deeply staining material. In addition to these figures, as we glance through the plates, we find further evidence. It is rather common to find lying between the attached arms an enlarged area which is chromatic. Figure 26 illustrates a case of this sort.

What has been described for the two arms of the third applies to all the other elements. Both the X's and the fourths are anchored to the chromocenter by narrow and usually transparent strands. From these observations it is clear that narrow and more or less transparent strands of protoplasm serve to anchor the banded portions of the elements to the chromocenter, and the conclusion seems warranted that these strands form, in part at least, the achromatic bridge between the arms seen in prophase and early metaphase stages. There is, however, associated with these strands some of the deeply staining chromocenter material. In text figures A7 and A8 two possible interpretations are shown diagrammatically. In A7, the two clear strands are shown connecting the two paired arms with a coating of chromocenter material on the outside. This interpretation would seem to fit well with text figures A1 and A4. In text figure A8, the chromocenter material is shown more or less localized or enclosed in a sac-like enlargement. This interpretation is suggested by text figures A2, A3, A5 and figure 26. In any event the main point to be stressed is that the banded or euchromatic area ends sharply and that the chromosome or arm is organized differently in the region adjacent to the spindle fiber. The character of the spindle fiber attachment zone, which lies within the chromocenter, is not known, at the present time.

DISCUSSION

Cytological and crossover maps

My study of the X chromosome showed a rather close correspondence between the morphological position of gene loci, on the salivary chromosome, and the crossover maps except at the extreme left-hand end (where gene loci are much farther apart than their crossover value would suggest,

due perhaps to the terminalization of chiasmata). Thus a break falling between lozenge (33.7) and vermilion (39.7) was actually 34 morphological units from the left-hand end, and the same general relationship is shown by other breaks. When we examine the cytological maps of the two arms of the third (figures 1 and 29) we are somewhat handicapped by a lack of exact genetic data for many breaks, nevertheless certain facts stand out sharply.

The genetic map of the third chromosome is made up of 105 crossover units, and as the spindle fiber is close to 48, the left arm has 9 less units than the right. The total length of the euchromatic areas of the left and right arms, as these appear on my original drawings is 640 millimeters, and thus, on the average, about 6 millimeters of map length should represent one crossover unit. If the left arm carries 48 units it should be 288 millimeters long and it actually measures 282. The right arm, in turn, should be 342 millimeters long and is actually 358. On the assumption that the banded areas of the two arms carry all of the genetically active material, then the actual lengths of the two arms corresponds well with the crossover units each is supposed to contain.

The cytological map of the left arm (figure 1) shows, in general, that the morphological position of gene loci are much farther to the left than their crossover value would indicate. Thus hairy (26.2) is placed close to 19 morphological units from the left end; thread (42.2) lies to the left of 33 morphologically and scarlet (44) apparently lies to the left of 37 units from the left-hand end. In passing it should be pointed out that the VI 34 and VI 22 breaks are to the left of roughoid (0.0) the gene locus farthest to the left of known mutations in the third chromosome. If the distal ends of the third are like the free end of the X, then this material to the left of roughoid will scarcely amount to one crossover unit.

In a similar way we observe that the topographical map of the right arm shows a similar pushing of gene loci away from the spindle fiber. Thus stripe (62.2) must lie at least 80 morphological units from the left-hand end of the whole third, and sooty (70) to the left of 85. On the other hand a break (VI 44) which is beyond claret, comes 103 units from the left end.

The greatest discrepancy, as we should expect, is between the crossover values and the morphological positions of scarlet and pink. Genetically these are 4 crossover units apart, but physically they must be at least 22 morphological units apart. This represents something over 1/5 of the total length of the banded areas and does not take into account, at all, the region of the chromocenter. Of course, crossover units simply express the frequency with which crossing over occurs between gene loci and it is clear that in the region between scarlet and pink there is less crossing over per morphological unit (4 in 130 millimeters on my original drawings) as

compared to the curled-stripe area where 10 crossover units are represented by 75 millimeters of length on my drawings. This all amounts to saying, what we have long known, that crossing over is reduced in the region of the spindle fiber, and now through a study of appropriate breaks by the salivary gland method it should be possible to gain exact information on this genetic problem.

The question now arises, why should there be a difference between the X and the third chromosome in the correspondence between cytological and crossover maps? It might be thought that it is the presence of two arms, as compared to the rod-like X. On the other hand, it must be

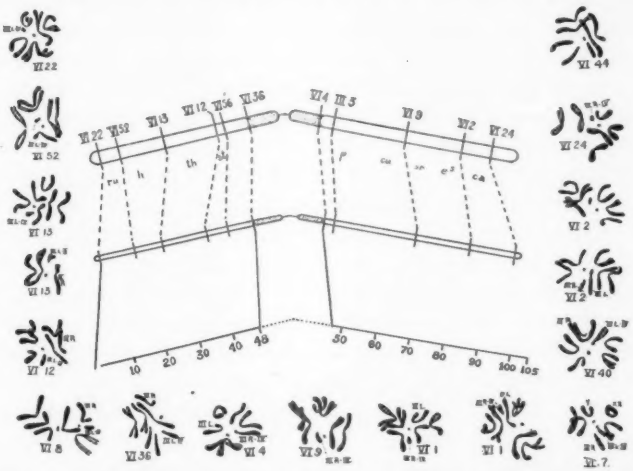

FIGURE B.—Around the periphery of this figure are a number of oogonial metaphase plates, taken from young adult flies which were heterozygous for the aberration indicated below the figures. In many instances the females were of the attached-X stock so that the two X's are joined and a Y chromosome is usually present. The upper central figure represents the third chromosome as it appears in oogonial metaphase plates. The middle central figure represents the salivary gland map while the lower is the crossover map.

remembered that nearly half of the X, next to the spindle fiber is made up of inert material so that physically the active gene-bearing material is far removed from the spindle fiber attachment zone. In the case of the third chromosome, the amount of inert chromatin is relatively small and it is possible that it is not sufficient to cancel the inhibiting effect of the spindle fibers. There are several other possible explanations of course.

A study was made of oogonial plates of a number of aberrations in which the points of breakage are well separated in order to compare the relative sizes of broken chromosomes in metaphase plates and salivary glands and the results are presented in text figure B, in the form of an old style

cytological map. At the outset it is well to emphasize, as both DOBZHANSKY and I have done in the past, the inherent difficulties in estimating the size of fragments relative to the chromosomes as a whole. If KAUFMANN (1934) is right, however, in his contention that there is a "differential contraction or extension of different segments" (p. 140) of chromosomes, even though we could draw and measure the length of pieces accurately, it would be of limited value because one would not know whether he was dealing with an extended or contracted phase of accordion-like chromosomes. In making estimates of the size of chromosome segments I have used linear dimensions in the main, and less value has been given to thickness.

In making a schematic outline of the third chromosome I have represented the two arms as uniform in diameter with only the primary constriction showing. Secondary constrictions have been reported for this element. According to DOBZHANSKY (1930) there are two, one in each arm not far removed from the primary. HEITZ (1933), however, maintains that only one arm has a real constriction, the other being formed presumably by the crossing of sister strands (see MULLER and PAINTER). And KAUFMANN (1934), who seems to be more constriction minded than the others, describes no less than five or six secondary constrictions for the whole third, if I interpret his figures and the text properly. In oogonial metaphase plates these secondary constrictions do not usually appear, and since the experts in these matters do not agree as to their position in the relatively large nerve cells, it seems wise to represent the chromosome much as it ordinarily appears. In any event, the main value of constrictions is their use as markers and in view of the development of the salivary gland chromosome technique where there are numerous physical markers and an abundance of constrictions which any one can see, it is quite clear that the number of secondary constrictions in prophase or metaphase chromosomes is now a matter of secondary importance and interest.

In text figure B the two arms of the third are shown as uniform in diameter because in salivary glands they exhibit this condition, and I quite agree with KAUFMANN (1934) that the tapered form seen in metaphase plates is largely the result of the prophase split and separation of sister strands. The right arm is represented as 10 percent longer than the left because there is actually this difference in the salivary gland and the crossover maps. Most investigators, in the past, have represented the two arms as equal in length, and in the contracted metaphase chromosome, a 10 percent difference would scarcely be apparent. It is interesting to note, however, that KAUFMANN (1934) shows the right arm somewhat longer than the left in prophase stages of giant nerve cells, where the chromosomes are relatively long. Below the metaphase chromosome is a greatly

reduced scheme of the salivary gland maps, with the inert material (stippled) lying about the spindle fiber. Below this the crossover map is so placed that the crossover units fall within the euchromatic areas of the salivary gland maps The positions of selected breaks are shown and there are camera lucida drawings of oogonia upon which the diagrams are, in part, based.

One outstanding feature of text figure B is the wide separation of gene loci for scarlet and pink on the metaphase chromosome. The proximal (to the spindle fiber) limit of scarlet is given by translocation VI 56, and the inner limit of pink is determined by the X-IV, III 3 break. No metaphase figures of the truncated right arm of the latter are available (all figures show it united with the fourth) but it involves 22 millimeters of the euchromatic area of the right arm, on my drawings, so I have estimated its size by measuring the amount of euchromatic material between the inert area of the left arm and the VI 56 break (which is 8 millimeters on the metaphase chromosome) and dividing this by half. Once the morphological positions for the loci of scarlet and pink are determined, there is a fair correspondence from these points running distally between the position of genes on metaphase and salivary chromosome maps. Unfortunately, many of our breaks have not been localized genetically and until this is done we are unable to plot many points on the crossover map.

That there is a discrepancy between cytological and crossover maps was first pointed out by Dr. MULLER and myself (1929) and one case cited was a break involving the third chromosome. Later the same year DOBZHANSKY (1929 and 1930) published a cytological map of the third chromosome based on 5 translocations and in these studies he calls attention to the wide separation of scarlet and of curled from the spindle fiber area. One of DOBZHANSKY's translocations (named "e") broke the left arm apparently, just where VI 36 and VI 14 did, and the position which DOBZHANSKY gave to scarlet corresponds very closely to the position I have arrived at, on the basis of the VI 56 break. The same correspondence is found in the general position of curled. I have placed pink farther away from the spindle fiber than DOBZHANSKY's maps suggest. Of course, these discrepancies are largely due to the fact that there is a very appreciable amount of the inert material in both arms of the third chromosome next to the spindle fiber, as HEITZ (1933) has already pointed out, and we turn now to a consideration of the character of the chromatin which lies outside of the euchromatic area.

The presence of genetically inert material in chromosomes was first demonstrated at this laboratory by the combined cytological and genetic work of Dr. H. J. MULLER and myself (PAINTER 1930, MULLER and PAINTER 1932) for the X chromosome of *D. melanogaster* and has since

been confirmed by a number of investigators. In my paper dealing with the X chromosome of the salivary gland, which appeared recently in this journal, it was shown that the active genetic region of the X is represented by the banded or euchromatic part and that the inert material is represented, in whole or in part, by the material of the chromocenter. The present work shows that the third chromosome is differentiated likewise into euchromatic areas and a chromocenter region and we interpret the latter just as we did in the case of the X.

Another line of evidence which indicates a similarity of behavior of the inert region of the X and the material adjacent to the spindle fiber in the third chromosome is the work of HEITZ, dealing with heteropycnosis, more especially his most recent work on *D. melanogaster*. Here he shows that in somatic mitoses the half of the X next to the spindle fiber area tends to remain condensed in late telaphases, the interphase and early prophases, when the more distal half is diffuse. The former is, of course, the genetically inert region and HEITZ cites the work of Dr. MULLER and myself as supporting the conclusion, which he had reached before our work had come to his attention, that heteropycnotic areas of chromosomes, in general, represent genetically inactive regions. This conclusion was not based on any direct genetic evidence but seems to have been an inference drawn from the passive behavior of the heteropycnotic areas, as these appear in plants.

HEITZ has shown that in addition to half of the X, and all of the Y when present, there are heteropycnotic areas adjacent to the spindle fiber region in both the second and third chromosomes. These observations have just been confirmed by KAUFMANN and also by one of my own graduate students (unpublished data). These heteropycnotic areas correspond to the area of the third chromosome which does not show the banded form.

In addition to his excellent work on *D. melanogaster*, HEITZ has studied heteropycnosis in several other species of Drosophila all of which show evidence of heteropycnotic areas for part or all of the elements. *D. virilis* shows the greatest amount of inert material, nearly a half of each chromosome except the fourths, and as a result there is an enormous chromocenter in this species. *D. hydei* shows the least, the inert material being confined to the sex chromosomes. Previous to his work on Diptera, HEITZ had made a series of studies on heteropycnosis in plants (1929, 1931) and had shown that the phenomena was quite common, especially in the mosses and that the areas may fall anywhere along the chromosome, that is, it is not necessarily associated with the spindle fiber region.

Regarding the nature and function of the inert chromatin we are at present in the dark. One point seems clear and that is the phenomena is not confined to or necessarily connected with the sex chromosomes and

that speculations (see for example, p. 350 of MULLER and PAINTER) which have this premise as a basis are ill-founded. In the salivary glands the inert region of the chromosomes is differently organized from the euchromatic part. There appears to be a core of transparent (matrical?) material to which the chromocenter material adheres in an irregular fashion and not in the orderly way the chromatin of the euchromatic areas is laid down. This picture seems to suggest that we are dealing either with undifferentiated chromatin or with excess material of some sort, but there are other possibilities. At a later time it is planned to consider the question of chromosome structure in detail. As this is written, it seems probable that the salivary gland chromosomes may be regarded as a chromonema lying straight. There are local deposits of chromatin along this (chromomeres); there has been a great hypertrophy of the matrix which has stretched the chromomeres laterally until they show as bands in what is essentially a tetrapartite structure (because each of the synapsed homologs is probably split before somatic synapsis).

Distribution of Breaks

PATTERSON and his associates isolated 36 translocations between the third and fourth chromosomes and 32 of these have been studied by the salivary gland method. The cases not analyzed fall within the genetic limits of scarlet and pink. When we consider the distribution of these breaks (figures 1 and 29) we find that 11 came within the euchromatic area of the left arm, 15 in the corresponding area of the right arm and 7 within the chromocenter. The breaks in the left arm are well distributed, though there is some crowding close to the chromocenter end (figure 1). In the right arm, by contrast there is a paucity of breaks until we reach the distal two thirds and here there is a marked tendency for the breaks to be bunched. No less than five, for example, fall within 3 morphological units in the region of 90 (figure 29).

If we consider the breaks from the genetic point of view we find altogether 17 out of the 36 cases come between the loci of scarlet and pink. This tendency for the breaks to occur in the region of the spindle fiber is not confined to the third. Nearly half of the translocations between the second and fourth chromosomes fall between purple and curved (PATTERSON et al 1934) and in a paper now in press Mr. STONE and I have shown that out of 14 translocations between the X and fourth, which are viable in the male, half fall within the chromocenter.

It will be apparent, at once, from the evidence discussed in the foregoing section, that while nearly half (17 out of 36) of the breaks fall between scarlet and pink, a distance of 4 out of a total of 105 crossover units, nevertheless these gene loci are physically widely separated, not

only by euchromatic material, but the inert and spindle fiber region as well. I have estimated that in the oogonial chromosome the distance separating these loci is not far from a fifth or fourth of the total length of the entire chromosome. Even with this separation we could not expect so many breaks here unless there is some definite reason for it. Again, in the right arm from about 48 to 68 there is only one break (which is a very involved case) while in the same region on the opposite side there are 6 breaks for a shorter physical distance. On the other hand, the distal portions of the right arm show a marked bunching of breaks not seen in the left arm. There must be some physical basis for this tendency for breaks to fall within limited areas while other areas are free from them.

At first sight it might be thought that certain regions of the third chromosome are more prone to undergo breakage than others, but the real explanation, probably, is to be sought in the orientation of the chromosome at the time the sperm was irradiated. This brings us to a discussion of a very illuminating phase of the salivary gland chromosome work, and that is the evidence for nuclear organization.

In the salivary gland each chromosome or arm is anchored to a common mass of material called a chromocenter. The latter name is relatively new to most of us, but the orientation indicated is nothing more than the persistence of the telophase arrangement. It so happens that in *D. melanogaster* there are heteropycnotic areas adjacent to the spindle fiber region of the large chromosomes and the fusion of this material makes a conspicuous marker. All of the evidence which we have on translocations points to close physical contact or proximity as a condition of mutual exchange during or immediately following irradiation. If it were possible to irradiate salivary glands and then in subsequent divisions to follow the chromosomes we should expect exchanges to be most numerous in the region of the chromocenter, or close to it, because this is the region of the most intimate physical contact. There can be doubt that at the beginning of spermiogenesis, in *D. melanogaster*, the chromosomes have the telophase orientation, as a result of the second maturation division. Should this arrangement persist, or tend to persist, in the mature sperm head, then we should expect to find what we have found indeed, a predominance of exchanges in the region of closest physical contact. To explain the bunching of breaks (or its absence) in more distal regions of the chromosome we would have to assume that the element tended to coil around in the sperm head in a rather definite way, so that it was close to or far away from the fourth. This general explanation has been used by my colleagues to explain the bunching of the numerous translocations which they obtained (see PATTERSON et al).

In studying the phenomena of somatic synapsis in the salivary gland

it has been quite evident that the telophase orientation of chromosomes plays a considerable role in the ability of homologous parts to get together. This is most clearly seen in exchanges involving the fourth chromosome. When, as is shown in figure 40, the free end of the fourth chromosome is attached to the third far removed from the spindle fiber region, synapsis of this area with its normal homolog in heterozygous larvae is very rare, while the spindle fiber ends regularly undergo synapsis. In a similar manner a good many cases have been studied in which there was an exchange beyond the euchromatic region of the fourth so that a long segment of the right arm of the third received its spindle fiber from the fourth and morphologically nothing more. Now when we study larvae hyperploid for such a segment of the third, we find that it regularly is associated with the paired fourths and only occasionally will there be a synapsis with homologous region of the normal third chromosomes. I have interpreted this to mean, not that the attraction between the spindle fiber area is greater than elsewhere along the chromosome, but that normally the spindle fiber areas of the fourth chromosomes are anchored to the same general region of the chromocenter and this physical contiguity favors the union. Whether or not the segment attached to the spindle fiber region of the fourth synapses with an homologous region of the normal chromosome will depend largely on how close they happen to lie in any given nucleus, and how many other elements lie between them. If such factors operate in the salivary gland cells where synapsis is a slow and progressive process, it seems inevitable that in meiosis we would have the same effect only in more pronounced form in these cells where changes go on more rapidly. Thus, it is possible that the attraction between the ends of chromosomes, such as BELLING has often described, has a simple physical basis.

On the whole, the writer is inclined to the view that the idea of nuclear orientation will prove helpful in our interpretation and understanding of chromosome behavior in meiosis, especially in forms in which there are aberrant elements, and that in the future this factor should be given due consideration in theoretical discussions concerning synapsis and crossing over.

From a theoretical standpoint the exchanges made between the third and fourth chromosomes in the region of the chromocenter are of extreme interest. Here we have either the replacement of one arm by a fourth forming a j, or a part of one arm is attached to the spindle fiber end of the fourth also forming a j. The questions which concern us here deal with the nature of the spindle fiber region and since we are unable to see this area clearly in salivary glands, or at least have not recognized, up to the present, these areas in the chromocenter, no direct evidence can be presented on

this point. At the same time we can not overlook the very obvious question of what happens when one arm of the third is replaced by a fourth chromosome. Since the displaced arm persists, it must have a spindle fiber attachment zone of its own. But how about the other arm? Does the spindle fiber of the fourth do double duty or does the other arm have a spindle fiber zone of its own? There is much evidence in favor of the latter point of view. Various aspects of these questions are discussed in a joint paper with Mr. STONE where we take up, in detail, the apparent fusion of the X and fourth chromosome.

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CHROMOSOME FUSION AND SPECIATION IN DROSOPHILAE

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Received September 3, 1934

In a paper which appeared recently Dr. J. T. PATTERSON, WILSON STONE and the Misses SARAH BEDICHEK and META SUCHE give a summary of their studies of a large number of translocations which they obtained in the spring of 1933. Most of the X-IV, III-IV, and II-IV translocations have now been analyzed cytologically, both by the usual oogonial metaphase plate and the new salivary gland methods, and it has been found that in many instances one whole arm of either the second or the third chromosome has been replaced by a fourth forming a j-shaped element, or, that the X and the fourth have united at their spindle fiber ends forming a j-shaped compound chromosome. Detailed descriptions of the exchanges involving the second and third and fourth chromosomes will be found elsewhere (PAINTER 1934b, 1935). The present paper deals with the "fusions" between the X and the fourth chromosomes. In the cases of the v-shaped second or third elements, it is not possible to determine whether there has been a mutual exchange with the fourth close to the spindle fiber attachment zone (that is, a mutual translocation) but in the case of the rod-like X and fourth there can be no such exchange if a j-shaped compound chromosome results. These cases are of very great theoretical interest both from the standpoint of the structure of chromosomes and of the behavior of chromosomes during speciation. It has been generally assumed by cytologists that number changes within a restricted group have come about largely through either a fusion of rod-shaped chromosomes or a fragmentation of j- or v-shaped elements, but little thought has been given to the question of what happens to the spindle fiber mechanism during these processes. These experimentally produced "fusions" offer an excellent opportunity for subjecting some of the questions involved to a critical analysis.

At this place the writers wish to express to our colleagues and associates our obligation and appreciation for the use of this material.

MATERIAL AND METHODS

In the original experiment, 14 translocations involving the X and fourth chromosomes, and viable in the male, were obtained. In 8 of these the gene string of the X appeared to be intact up to and including the normal allele of bobbed. The genetic data are presented in an article elsewhere by STONE (1934). A cytological study of these 8 cases shows that

they fall into three classes, (a) simple unions at the spindle fiber ends of the X and fourth forming a j-shaped element, (b) multiple unions of the X, the fourth and a segment from the right arm of the third forming a compound and essentially a Y-shaped chromosome, and (c) possibly a simple translocation during which the euchromatic area of the fourth becomes attached to the side of the X, in the inert region, or directly to the spindle fiber attachment zone.

The cytological study was made by the aceto-carmine method applied to the ovaries of young adult flies and to the salivary glands of old larvae.

Before we describe our cytological findings, it will be necessary to briefly review the general set-up found in the salivary glands and the normal topography of the X and fourth chromosomes even though this involves some repetition of facts already described in papers which are in press as this is written.

In the salivary glands the chromosomes are differentiated morphologically into euchromatic or banded areas, which carry all or nearly all of the active genetic material, and heterochromatic areas composed of genetically relatively inactive material. This heterochromatin which is carried by the X's, the Y, the second and the third chromosomes, is not organized into separate (visible) distinct parts of the individual chromosomes but unites to form a common mass of deeply-staining material which HEITZ (1933) has termed a chromocenter. The euchromatic parts of the chromosomes are connected to the chromocenter, in part at least, by thin protoplasmic processes which, directly or indirectly, connect together the two arms of the second or the third chromosomes, respectively. It is highly important, for what follows, to realize that the two arms of the second or the third element enter or are connected to the chromocenter separately, and that there is ordinarily no visible connection between the two except the deeply staining heterochromatin which is shared in common with the other elements.

The orientation shown in figure 1 is arbitrary and schematic, but the pattern of the euchromatic areas of the several elements in contact with the chromocenter, is accurately represented. In all probability the various arms or elements are all attached to one side of the chromocenter rather close together. One can visualize the probable orientation as a telophase arrangement, for the inert chromatin happens to lie at the spindle fiber ends of the chromosomes. In any given preparation the position assumed by the elements of the nucleus is the chance result of how these happen to lie with relation to the cover glass at the time the nucleus was crushed. Thus the spindle fiber ends of the third chromosome, for example, may be attached to the chromocenter close together, or they may be widely separated.

In old larvae, as PAINTER (1933a, 1934b) has shown, homologous chromosomes undergo somatic synapsis and form one apparent element. They are so represented in figure 1, but the double connections within the chromocenter indicate their dual character. Somatic synapsis is of value to us in this study because when you have a normal and an aberrant homolog united, it is easy to determine if any bands from the euchromatic area are missing.

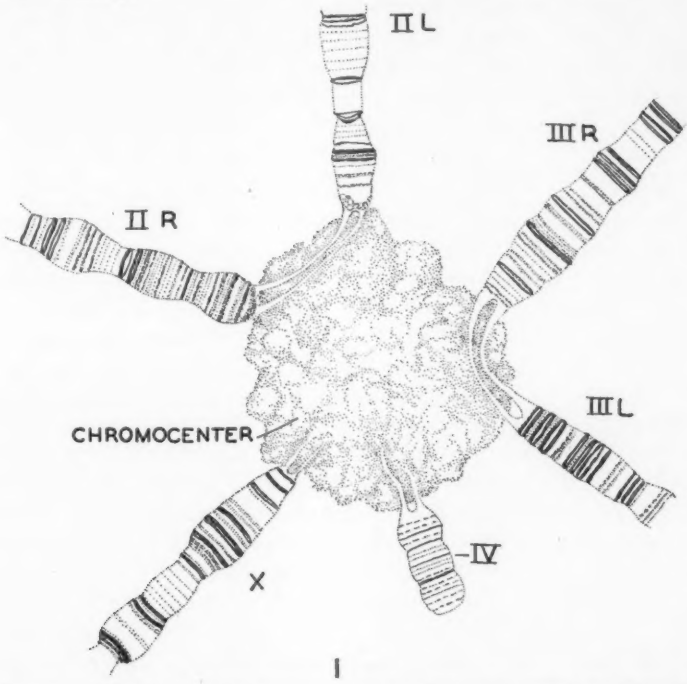

FIGURE 1.—This is a semi-diagrammatic sketch showing the general orientation of the chromosomes about the chromocenter in the salivary gland. The pattern of the ends of the various elements or arms is accurately represented for the region where they enter the chromocenter. The clear protoplasmic strands connecting the elements with the chromocenter also exist, but the way the two arms of the v-shaped II or III are connected is diagrammatic.

The normal topography of the X and fourth chromosomes, insofar as we need to consider this phase, is given in figure 1.

SIMPLE UNIONS (FUSIONS)

We have altogether 5 cases of this type, 4 of which will be considered in detail. Complete data for one case (T1–4,15A) is lacking because the stock was lost before the genetic tests were complete or cytological draw-

ings made. Fortunately, it was the first case examined cytologically and the one which gave us the key as to the nature of these experimentally produced compound chromosomes. From the genetic standpoint all these cases behave alike in the following respects: (a) All show crossing over beyond carnation and no hyper- or hypoploids live when they are tested to forked, carnation, bobbed and eyeless. In other stocks involving short pieces of the X–IV, for example, T1–4, 13A, such aneuploid flies appear. This indicates that the union is beyond bobbed. (b) All show complete linkage between eyeless and bent with no hyperploid phenomenon, (c) As compared to the normal the reduction in crossing over is slight in all cases. (For full genetic data see paper by WILSON STONE.)

CYTOLOGICAL EVIDENCE

T1–4, 5A

Figure 2 is an oogonial metaphase plate taken from a fly which was heterozygous for the union between the X and the fourth. This individual carried a Y chromosome which is labelled. The figure shows one normal fourth, one normal X, and an X to which a fourth is attached forming a j-shaped element. Figure 3 is taken from a female homozygous for this aberration, and one sees that both X's have a short arm the size of normal fourth chromosomes attached to their spindle fiber ends. No free fourths are seen in the cell and the diploid number of this fly is 6 and not 8 chromosomes. A study of the salivary glands of old female larvae heterozygous for this aberration (figure 4) shows that both the paired X's and fourths are morphologically intact and are attached to the chromocenter separately. There is no suggestion of any direct union between the two. The patterns of the two X's correspond throughout their length (only the spindle fiber ends are shown) and it is quite certain that nothing is missing from the euchromatic area of either homolog. The paired fourths, in like manner, are paired throughout their lengths and nothing is missing from the euchromatic areas. Any connection between the X and the fourth must be hidden by the chromocenter, just as in the case of the two arms of the second or the third.

T1–4, 6A

Figure 5 is an oogonial metaphase plate taken from a fly which was heterozygous for this fusion. It will be seen that there is one free fourth and one attached to the spindle fiber end of the X. (We use the term spindle fiber end to indicate that part of the euchromatic area nearest the chromocenter.) Figure 6 is from a female larva heterozygous for this aberration. This figure is of unusual interest as one can see the complete morphological similarity of the normal and aberrant homologs. The figure

shows the paired X's and fourths as they lay on the slide. The X's have pulled free, a little, from the chromocenter but they were attached separately from the fourths. In all respects this case is like the preceding cytologically.

FIGURES 2 to 22.—The magnification of the oogonial plates is either 2400×, or 1800×, at the eye piece. Salivary gland drawings represented 900×, at the eye piece. All drawings were made by the use of a camera lucida at table level, and have been reduced about one-fourth in reproduction.

FIGURES 2 and 3 are oogonial metaphase plates of flies which were hetero- and homozygous respectively for T1–4, 5A. Figure 4 shows the condition of the X's and fourths of this translocation in salivary glands. Figure 5 is an oogonial plate from a heterozygous fly, and figure 6 a salivary gland preparation from a heterozygous larva of T1–4, 6A. Figures 7 and 8 are taken from individuals heterozygous for T1–4, 7A. Figures 9 and 10 were taken from heterozygous individuals for T1–4, 11A. Figures 11 to 14 are oogonial chromosomes from flies heterozygous for T1–4, 3A. Figures 15 to 17 were taken from salivary gland material. Figures 18 and 19 are oogonial plates from flies hetero- and homozygous for T1–4, 2A. Figure 20 is from the salivary gland. Figures 21 and 22, are from the ovary and the salivary gland of individuals heterozygous for T1–4, 14A.

T1–4, 7A

Figure 7 is from a female heterozygous for this fusion and shows a normal fourth and an X with a fourth attached to its spindle fiber end. Figure 8 is from a heterozygous female larva. In this figure the X's and fourths are quite intact in the euchromatic area; they happen to lie close together but are connected separately to the chromocenter.

T1–4, 11A

Figure 9 is an oogonial metaphase plate taken from a fly which was heterozygous for this aberration. One of the fourth chromosomes is attached to the X. Figure 10, taken from a heterozygous female larva, shows the X's morphologically intact, as are the fourths, and these are attached separately to the chromocenter region. Incidentally some of the other elements are indicated. This aberration is cytologically just like the others considered above.

MULTIPLE UNIONS

T1–4, 3A

We have only one case of this type. Genetically it behaves as a simple fusion, like the cases described in the foregoing section, but salivary gland material shows that it is a complex case. Figures 11 to 14 are taken from oogonial metaphase plates of flies heterozygous for the rearrangement. A glance at the figures shows that one of the X's is j-shaped but that the short arm is much larger than the single fourth chromosome found in the figures. When this case was first examined in oogonia it was thought that some of the inert material from the X had in some way become associated with the fourth giving the large size. But when the salivary glands of old larvae heterozygous for this aberration were examined, it was found that the fourth was united with a short section from the right arm of the third chromosome. Figure 15, taken from a heterozygous female larva showed the paired X's (not illustrated in this figure) completely intact and attached independently to the chromocenter. The paired fourths, on the other hand, had attached to one side of one of them, right at the margin of the chromocenter, a short piece of another chromosome which was recognized at once as the spindle fiber end of the right arm of the third. Note in figure 15 that the tip of the right arm and one of the fourths are closely joined at the spindle fiber ends of the two elements. Figure 16 shows the ends of the X's below. They are normal throughout their lengths and are attached separately to the chromocenter. Above and to the left are the normal synapsed right arms of the third chromosome which become very broad where the hyperploid segment of the third joins with them. To the right the two fourths are shown attached, apparently, to

the spindle fiber ends of the right arm. Figure 17 shows essentially the same relations as figure 16, but the right arms of the paired thirds are bent over obscuring many details. The two essential features to be noted from these figures are first, that the X's and the fourths are attached separately to the chromocenter and second, that a piece of the third chromosome that is a part of the euchromatic area is closely joined to the spindle fiber end of one of the fourth chromosomes. This case has great theoretical interest, as we shall show in our discussion.

OTHER CASES

Under this heading we have to describe two aberrations which genetically show the X and fourth completely linked, as in the simple fusions, but in which crossing over is much reduced

T1–4, 2A

Figures 19 and 18 show heterozygous and homozygous oogonial plates of this aberration. In the latter figure, the fly was hyperploid for the fourth. In both of these figures the fourth chromosome appears to be attached to the side of the tip of the X, and the two are not joined at the spindle fiber ends to form a distinct j, as in the cases previously considered. It may be remarked that these figures were drawn and the condition noted before the explanation was apparent. When salivary gland material of heterozygous larvae was studied, at first glance it looked just like the other cases. On further study it was found that in many cells the X and fourth were closely joined at the edge of the chromocenter. Figure 20 illustrates such a case. (Of course, in mashing out the nucleus this association was frequently disturbed, as we might expect.)

T1–4, 14A

Figure 21 is an oogonial plate of a fly heterozygous for this union. We note that an X has a fourth attached to it by a chromatic bridge. Figure 22 is from the salivary gland of a heterozygous larva and shows that one of the synapsed X's is attached, at the edge of the chromocenter, to one of the paired fourth chromosomes.

DISCUSSION

The facts presented in this study show that both genetically and cytologically we are dealing with two different types of aberrations. They are all alike in that the gene strings of both the X and the fourth are intact and completely linked, likewise in that the euchromatic areas of both elements are morphologically intact. They are unlike in that the frequency of crossing over is much higher (and similar in amount) in T1–4, 3A –5, –6, –7 and –11 than in –2 and –14, where we find a sharp reduction. Cytologically these two classes differ in the salivary gland in the way the X

and fourth are associated. In the first type (–3, –5, et cetera) the X and the fourth are attached to the chromocenter separately, and the connection between the two is, in some way, accomplished within this structure. The second type (–2, and –14) shows a much more intimate association between the X and fourth in that the two are frequently seen lying in contact on the edge of the chromocenter (they are sometimes separated when the nucleus is crushed). We think that these differences in the way the two chromosomes are associated explain the difference in the rate of crossing over of the two types, as explained below.

In his cytological study of the third chromosome PAINTER found the same two classes of exchanges between the third and fourth chromosomes, in the region of the chromocenter, as we have described in the foregoing pages. In one type, the whole arm of the third chromosome is simply replaced by an intact fourth, and such cases are entirely comparable with the simple unions of the X and the fourth, not only cytologically but genetically, because Miss SUCHE has found that crossing over is reduced very little by the fusion process (unpublished data). In the second type the right arm is broken just at the edge of the chromocenter and is attached to the fourth somewhere between the euchromatic arm of the latter and its spindle fiber. These are similar genetically to our T–2A and –14A in that Miss SUCHE (unpublished data) finds a sharp reduction in the amount of crossing over.

One of the most interesting features about the salivary gland is the fact that the two arms of the v-shaped autosomes enter the deeply-staining chromocenter separately. At or near the point where they are attached, the banded form ends; and the arm is visible for a short distance within the chromocenter (in favorable cases) as a thin protoplasmic strand of, presumably, matrical substance. We know little, as yet, about the nature of the connection between the two arms; some possibilities have been considered by PAINTER in his study on the third chromosome, but whatever its character it is obviously quite different morphologically from the active genetic or euchromatic (banded) part. This brings us to a new and clearer concept of the nature of the chromatic bridge (achromatic with certain stains) which connects the two arms of these v-shaped autosomes. This subject interests us here very directly because the study of the simple unions of the X and fourth chromosomes, induced experimentally, shows that morphologically (as seen both in ovaries and in the salivary glands) the relation between the joined X's and fourths is the same as that which exists between the two arms of the v-shaped autosomes, and any conclusions which we may reach concerning the origin of the j-shaped X and fourth will probably apply to the large v-shaped autosomes of *D. melanogaster* and presumably, to v- and j-shaped chromosomes in general.

We have already emphasized the fact that in these simple unions of the X and fourth chromosomes both elements are completely intact genetically and so far as can be observed, cytologically. In oogonial metaphase plates both the X and the fourth are normal-sized and differ in no visible way from the normal homologs except that they are joined at their spindle fiber ends as salivary gland studies show. The question now comes to the fore, what has happened to the spindle fiber mechanisms of the elements, are these intact also, or has one of them been lost?

Unfortunately we are not able to answer the above questions, as yet, in *D. melanogaster*. The presence of the large amount of deeply-staining inert chromocenter material obscures the details and no way has been found to get around this difficulty. In all probability the cytological answer must be sought in species of Drosophila in which there is little inert material and a survey of available species with this point, among others, in view is now in progress at this laboratory. In the meantime, we shall consider some theoretical ways in which the X and fourth may be joined and make pertinent comments about certain combinations.

In figure 23 we have represented diagrammatically various ways the X and fourth may have united. We are assuming that the spindle fiber mechanism is terminal and is connected to the euchromatic area in some such way as is shown in the diagrams.

Diagram A assumes that X and fourth were joined, through irradiation, in the chromocenter region distal to the spindle fiber mechanism, but instead of separating with an exchange of spindle fibers, which would give an ordinary mutual translocation, they remain associated in some one of the three ways indicated by diagrams A1, 2, or 3.

Diagrams B and C (which are alike in principle) assume that the spindle fiber attachment zone of one element becomes attached to the connecting strand of the other in the region of the chromocenter, but distal to the spindle fiber. The various sequelae are shown

Diagram D assumes that the X and fourth have joined at the spindle fiber attachment zones and that, as a result, both elements lose nothing during the union.

Of the various theoretical possibilities we have definite evidence at this laboratory, for A2 in two translocations involving the third and fourth chromosomes. It is probable, as we shall show below, that the right-hand tip of the third and the fourth chromosomes in T1–4, 3A are associated essentially as is shown in A2. At the present time we are inclined to interpret the association of the X and fourth in T1–4A, 3, 5, 6, 7, and 11 as simple unions involving no loss as in diagram D and T1–4, 2A and 14 as like A2 or A3. The evidence will now be briefly reviewed.

The breaks in the third chromosome referred to are described by

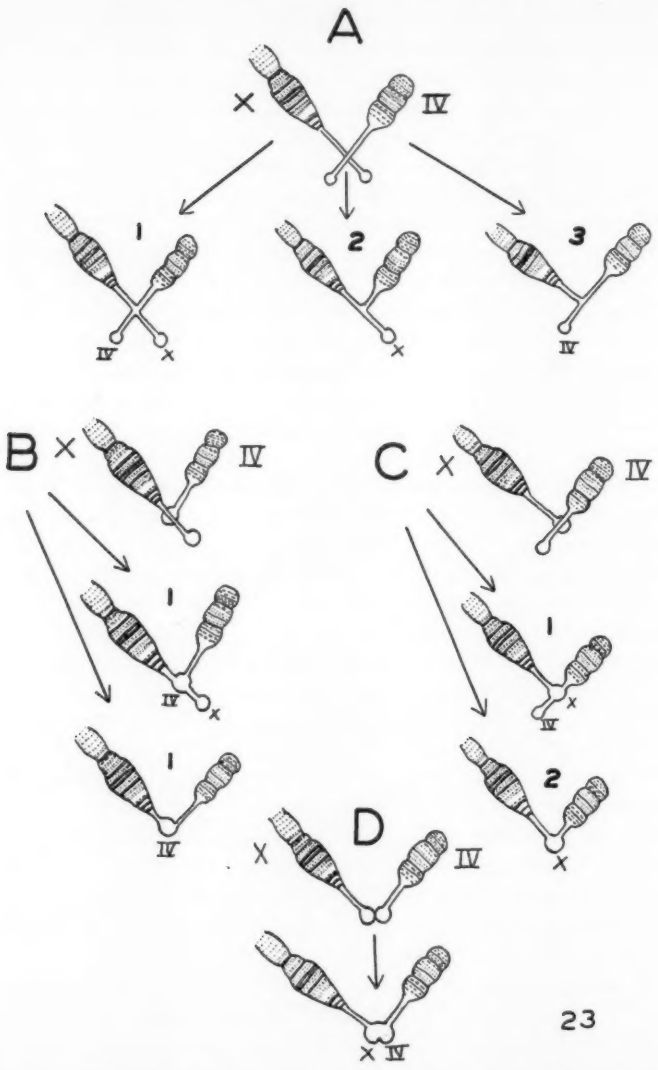

FIGURE 23.—This set of diagrams represent some of the most obvious ways v- or j-shaped chromosomes may have arisen from rod-like elements. For details see the text.

PAINTER under VI 27 and VI 4. The euchromatic area of the right arm of the third was attached to the spindle fiber end of the fourth beyond its euchromatic area. In oogonial metaphase plates there were two j-shaped

elements. One was the III R–IV compound, the short arm of the j being the size of a normal fourth. The other j was formed by the left arm of the third and the inert material which belonged to the right arm but which was left in its normal position when the euchromatic area of the right arm became attached to the fourth chromosome. In salivary gland material the right arm of the third is attached to the fourth just at the edge of the chromocenter, and since the spindle fiber mechanism of the fourth lies distal to this point, this III R–IV compound chromosome must be essentially a Y as in diagram A3.

In discussing the T1–4, 3A aberration, at this point, we are confining our remarks to the nature of the association between the tip end of the right arm of the third and the fourth chromosome. The association of this compound with the X will be taken up later. Figure 15 shows that the tip of the right arm is intimately connected with the spindle fiber end of one of the synapsed fourth chromosomes. This means that the contact between the two is right at the edge of the chromocenter and either that both elements have processes running into the chromocenter or that one or the other has lost its chromocenter connection. On this point the evidence favors the latter point of view and indeed suggests that it is the spindle fiber end of the right arm which is doing double duty. In the first place, oogonial metaphase plates (figures 11 to 14) show that the short arm (made up of the IIIR and IV material) is far larger than we would expect judging from the size of these in salivary glands. The volume of the combined euchromatic areas of the two can scarcely be more than three times that of the normal fourth. The knob on the end of the X is far in excess of this and is just about the size it should be if the inert material carried by the right arm were incorporated. This would be the case, were the spindle fiber of the third retained.

Again, the right-hand arms of the normal third chromosomes usually synapse with the fragment of the third and the synapsis of these elements would be greatly facilitated if they had homologous spindle fiber attachments. (PAINTER, in his study on the third chromosome has pointed out that chromosomes or arms are probably attached to a definite part of the chromocenter and that homologous spindle fiber zones must be close together. See PAINTER 1935a, p. 325.)

And finally, STONE has found that the fourth component may drop off while the III R component retains its association with the X, which suggests that the fourth is loosely attached to the former.

If the two elements of the III R-IV complex are joined at their spindle fiber ends, then in their association with the X they must make a y-shaped or forked chromosome complex. This is not visible, however, in oogonial metaphase plates, a fact which should not be surprising. It is well known

that at metaphase all chromosomes are split into two daughter elements and yet they appear as one. Under the circumstances we would not expect to see the fork between the short III R and IV elements. Attention should be called also to the translocation which DOBZHANSKY and STURTEVANT (1931) have reported, in which, judging from the genetic data, a piece of the second chromosome was attached to the side of the third chromosome in the region of hairy. In metaphase plates they were unable to see this association, but it should be visible by the salivary gland method.

In concluding that the connection between the X and fourth chromosomes in T1–4, 2A and 14 are different from the other X–IV unions considered below, we are guided by a number of considerations. First, in oogonial metaphase plates it is noted that the fourth seems to be attached to the side of the X (figures 18, 19 and 21) close to the tip and not directly to the tip. In the second place, in salivary gland material, we find the X and fourth, as a rule, connected right at the edge of the chromocenter, a fact which we have stressed. This is the same sort of association which PAINTER found in VI 27 and VI 4, and like these the T1–4, 2A and 14 show a marked reduction of crossing over as compared to simple fusions. T1–4, 2 and 14 must therefore be essentially y-shaped chromosomes like diagrams A2 or A3.

We have now to consider the evidence which causes us to believe that the simple unions of the X and fourth chromosomes are real fusions during which the spindle fiber areas of neither is lost. It may be said at once that we have no direct cytological evidence to offer on this point and that we are influenced by indirect evidence and by general cytological considerations.

As we have already outlined in connection with figure 23, there are three general ways in which the X and the fourth might have united, illustrated by A, B, or C, and D. In the first case we would have an X-like figure or, by derivation, a y. In the second a y-like arrangement or secondarily a v. In the third a v with two spindle fibers would result. The first two of these possibilities seem less likely because we actually have cases like A2 or A3 in which there is a great reduction of crossing over, and were the union of the X and fourth like B or C, we would expect the same effect. This leaves D as the most likely method of union, as we now interpret our results.[1]

[1] The theoretical interpretations given in figure 23 are based on the assumption that both the X and fourth chromosomes are rod-shaped, but recently KAUFMANN (J. Morph. 56, 1934) and PROKOFIEWA (Z. Zellf. Mic. Ana. 22, 1935) have figured and described the X as essentially j-shaped and KAUFMANN thinks that the fourth also has this form. Granting the correctness of the interpretation given for the tiny terminal chromomeres seen with special technique (though other explanations are possible) then our cases -5, -6, -7 and -11 might have resulted from mutual translocations, but cases -2, -3 and -14 cannot be explained in this way.

It seems probable that two of the three possible types of union between rod-shaped chromosomes have been obtained experimentally. It now is pertinent to consider the structure of the v-shaped second and third chromosome. As far as the cytological evidence is concerned, we have no direct evidence to offer; they may have arisen in any one of the three ways we have considered. It will be possible, however, to test out the nature of the connection between the two arms with breaks we now have, and the building up of the necessary testing stocks is in progress at this laboratory. So far as we know, no one has ever made a critical experiment covering this point.

Parenthetically, it may be remarked that crossing over is much less in the euchromatic area adjacent to the spindle fiber, per morphological unit of the salivary gland chromosome in the case of the second and third chromosomes than it is in the case of the X. PAINTER suggested that the presence of such a large amount of inert material in the latter may cancel out the inhibiting effect of the spindle fiber region. It is possible, however, that the second and third chromosomes are essentially y-shaped in structure.

The theoretical cytological background which leads us to believe that the compound X and fourth chromosomes have two spindle fiber attachments will be briefly filled in. It has been quite generally assumed, by cytologists, that chromosome numbers have been changed by the fusion of rod-shaped elements or a fragmentation of v- or j-shaped chromosomes. Scattered through the literature there are many cases which support these ideas (see WILSON 1928, or REUTER 1930, p. 89, for examples). But we have known nothing about what happened to the spindle fiber mechanism when fragmentation or fusion occurred. In the meantime, experimentally-induced fragmentation following irradiation has taught us that regardless of how the bio-physicists may interpret the nature of the spindle fibers in dividing cells (be they optical illusions or what not), the fact remains that unless a piece of or a whole chromosome, does not carry what we may non-committally call a "spindle fiber attachment zone" it is lost sooner or later from the chromosome complex. If fragmentation has occurred in nature, we must assume that the spindle fiber attachment zone was divided and was essentially plurivalent in the first place. (We regard the *de novo* origin of the attachment zone as very improbable.)

The presence of two spindle fiber attachment zones on one chromosome raises the question of whether this would make a stable complex. We share the opinion already expressed by MULLER and PAINTER (1932), NAVASHIN (1932) and others, and supported by the recent work of Mrs. L. V. MORGAN (1933), that when the attachment zones are far apart such a compound chromosome would probably break down. On the other hand, we

must assume a definite structural polarity within the chromosome and if the two centers were properly orientated with regard to this and close together, the compound element would probably be stable. As a matter of fact, we appear to have in the attached-X race of Drosophila, a compound element such as we are postulating and it is to be noted (ANDERSON 1925) that crossing over is not appreciably reduced, just as STONE finds in T1–4, 5A–6A et cetera. We must also remember the classic case of Ascaris in which there are possibly as many spindle fiber attachment zones in the chromosomes of germ and early cleavage cells as there are dot-like elements in the somatic tissue.

One further comment may be pertinent, and that is, in our discussions, we are apt to think in diagrammatic terms and may thereby get far away from the physical structure of the chromosome. Thus, while in plants we have many instances of attachment zones which appear to be discrete bodies (see TRANKOWSKY 1930, for instances, and the literature) nevertheless, botanical cytology is filled with careful drawings showing broad attachment zones with a whole tuft of fibers running from the chromosome towards the two poles. Here the attachment zone is certainly very long and is, presumably, capable of subdivision. In animals, in general, the attachment zone seems to be more localized, but after all it may be capable of fusion or of subdivisions and still remain functional.

From the phylogenetic standpoint the assumption of plurivalent spindle fiber attachment zones (it makes no difference whether a general area is divisible, or is made up of several closely connected centers) would readily explain what has been often found when the chromosomes of a restricted group of animals are studied. While most species have the same or nearly the same number of chromosomes, here and there we encounter a species which has a much higher number than we would expect. In addition, CROSS (1931 and unpublished data) has found that the species with the higher numbers may have as many v- and j- shaped elements as the generic type, showing that the higher number could not have been derived by a simple splitting of v- or j-shaped elements. The article by Dr. CROSS cites much pertinent literature.

T1–4, 3A is interesting from the phylogenetic point of view. In the homozygous female the genes of the duplication of the third chromosome are in the tetraploid condition, yet these females are perfectly viable and fertile. If this case is one that involves two spindle fibers, this fragment could later become free and furnish a new spindle fiber attachment zone to which, by translocation, other chromatin could be added. This would raise the chromosome number of the race.

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STUDIES ON UNSTABLE CHARACTERS IN PETUNIA.

I. THE EXTREME FLOWER TYPES OF THE UNSTABLE RACE WITH MOSAIC COLOR PATTERNS

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Received December 7, 1934

INTRODUCTION

Since the publication of DE VRIES' "Mutationstheorie" numerous cases of unstable characters have been examined. The first account of the inheritance of the striking somatic variations so commonly found in variegated plants was given by DE VRIES (1901) in his discussion of eversporting varieties. At the present day thanks to the results obtained by EMERSON (1917), EYSTER (1928), DEMEREC (1928, 1931), OEHLKERS (1930) and others the explanation of the behavior of unstable characters on the basis of the theory of unstable (that is, frequently mutating or "mutable") genes seems to be the most probable.

The unstable characters observed in plants concern mostly the distribution of pigmentation in leaves, flowers and other plant organs. In the unstable race of *Petunia violacea* Lindl. we have to deal with a large range of variation not only with regard to the color patterns on petals but also with regard to other characters both physiological and morphological. All those characters seem to be determined by one unstable gene with pleiotropic effect.

The extreme types of the unstable race of *Petunia violacea* differ in the following characters:

1. in the sizes of flowers
2. in the distribution and the amount of pigment in the flowers
3. in the sizes of the epidermal cells of petals
4. in the percent of abortive pollen grains
5. in the percent of germinating seeds.

Between the extremes are many gradations regarding all just-mentioned characters. The larger the flowers are the more pigment they contain, that is, the more numerous are the patches and the more densely they are distributed on the surface of the petals. Corresponding to the increase of the sizes of flowers the percent of germinating seeds increases and the number of abortive pollen grains becomes less numerous. Each flower type may reproduce the whole range of variation characteristic of the unstable race but it gives a majority of plants like itself, or in neighbouring classes of mosaic, with relatively fewer in classes remote from parental type. The selection of the extreme flower types results in the isolation of particular lines differing from one another in the frequency of mutation.

The variability of the unstable race reveals itself in the occurrence of different flower types not only in different plants but also in one individual (sectorial flowers and sectorial plants). The variability of particular shoots may also be a continuous one when the flowers of related types succeed one another. The experiments on crossing of the unstable race with the variety "Admiration" (belonging to *P. violacea*) show that the mosaic distribution of pigment is a recessive character, the uniform distribution being a dominant one. These characters are transmitted independently of the violet color, the sizes of flowers and the distribution of pigment in the form of stripes characteristic of the variety "Admiration". The unstable race has been also crossed with white *Petunia nyctaginiflora* and the F_1 plants have had also self-colored flowers. In this paper I give a detailed description of the two extreme types of plants of the unstable race leaving to further publications the presentation of the data concerning the selection experiments as well as those regarding the behavior of chimeral plants.

THE FLOWER TYPES

The differences in sizes between the extreme flower types are marked. The smallest flowers have about 1.5 cm in diameter while the largest ones about 5.0 cm. Some flowers of the unstable race are shown in figure 1. We see the extreme types (figure 1A and figure 1F) and some intermediate forms (figure 1B-E). The amount of purple pigment varies with the sizes of flowers. In small flowers it is distributed in the form of spots and patches in the epidermis while the large flowers are self-colored. The larger the flower, the larger on an average are also the purple patches. The correlation between the amount of pigment and the sizes of flowers is a significant one and the coefficient of correlation equals 0.837 (MALINOWSKI and SMÓLSKA 1932). The smallest spots on the petals of mosaic flowers have dilute purple color, the larger ones being always darker. In the small flowers the purple spots appear chiefly at the midrib of the flower lobes. In larger ones the middle part of the petals is self-colored, the margins being spotted and patched. As I have pointed out, the sizes of spots are proportionate to the sizes of petals. In larger petals they fuse with one another and between large patches so produced narrow and irregular spaces of white or light purple color are seen.The narrowest spaces are of light purple color, the broader ones being white. Figures 2 and 3 show the distribution of white, purple and light purple areas on small, intermediate and large petals. In these figures we see the drawings representing the halves of flower lobes with exact distribution of purple and light purple spots. The purple spots are shown in black, the light purple ones in grey.

The anthocyanin is confined to the epidermal cells of flowers.

ANATOMICAL RESEARCHES

The epidermis of petals on both the upper and the lower surface is composed of larger cells in large self-purple flowers than in small white ones. In mosaic flowers the epidermis of white area is composed of smaller cells than that of purple area. The epidermal cells of purple area and those of

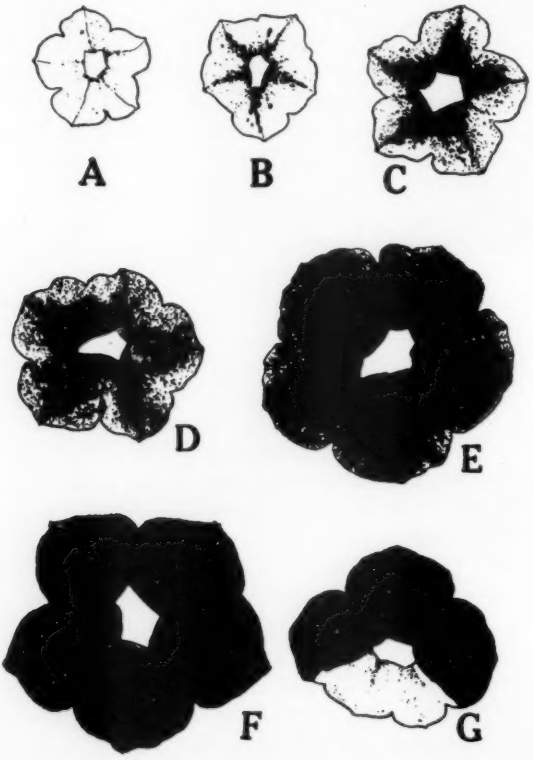

FIGURE 1.—Seven flowers of the unstable race. A and F—the extreme types; B, C, D, E—intermediate forms; G—sectorial chimera. Natural sizes.

the self-purple flowers are of papillose type as far as the epidermis of the upper surface is concerned. On the lower surface of petals in both extreme types of flowers the epidermis is composed of cells with irregularly lobed outline (figure 4). Besides this the cell walls of the lower surface form folds or "loops" directed to the inside of the cell. Such loops may also be observed in the upper epidermis of the self-purple flowers but in this case they are much smaller and in our preparations (figure 4) they look like

minute protuberances. In the upper epidermis of the white areas of the mosaic flowers the protuberances are missing.

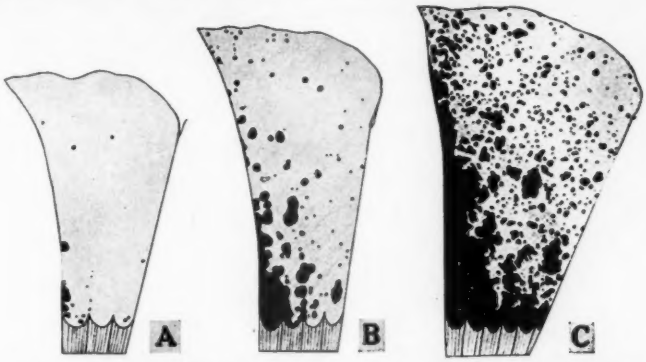

FIGURE 2.—Halves of petals of three flowers shown in figure 1, denoted by the letters A, B, C. The letters in both figures (i.e. figures 1 and 2) refer to the same flowers. Dark purple spots are shown in black, light purple ones—in grey.

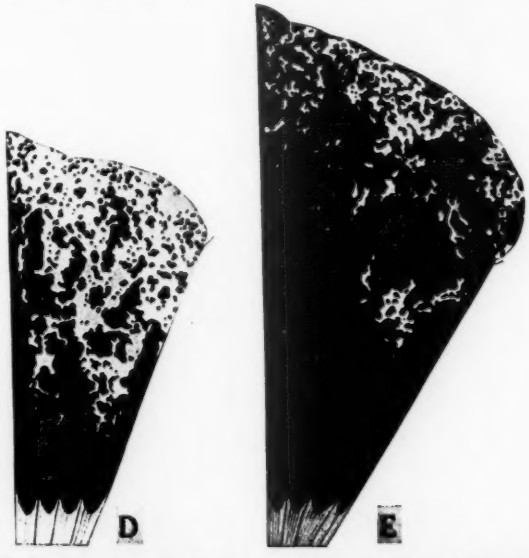

FIGURE 3.—Halves of petals of the flowers shown in figure 1 and marked by the letters D, E. The letters in both figures (i.e. figures 1 and 3) refer to the same flowers. Dark purple spots are shown in black, light purple ones in grey.

Let us pass now to the anatomy of small purple spots of the mosaic flowers. In figure 5c, a spot is shown composed of 8 purple cells. Their

walls possess small protuberances. This group of 8 cells is surrounded by smaller and distinctly lighter ones. The walls of these cells have not any

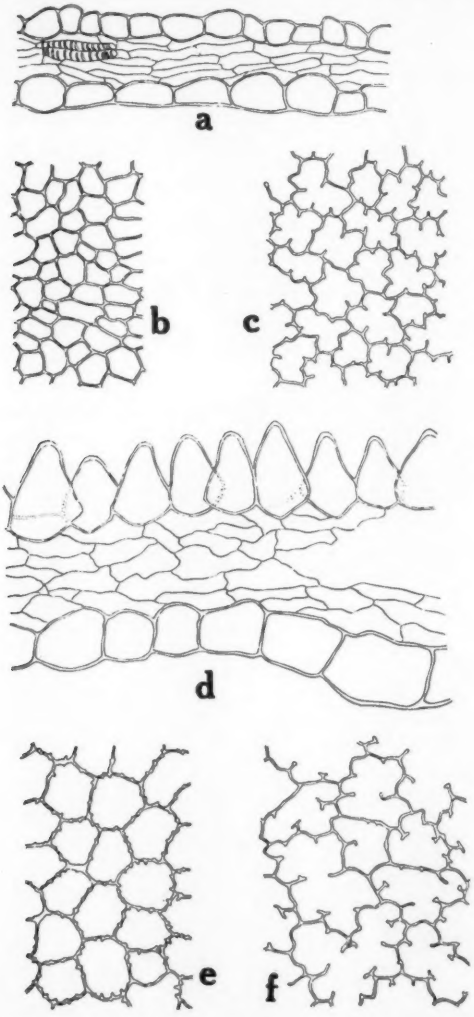

FIGURE 4.—Anatomy of petals of two extreme flower types. a, b, c—cross section (a) and the upper (b) and lower (c) epidermal cells of a small mosaic flower; d, e, f—cross section (d) and the upper (e) and lower (f) epidermal cells of a large self-purple flower.

protuberances. There are one or two layers (sometimes more) of such intermediate cells and around these layers we find small white cells char-

acteristic of the white areas of petals. Every purple spot either small or large is surrounded by a layer of intermediate cells. Such cells are distinctly lighter and a little smaller than the purple ones. In the flowers with white areas confined to small irregular spaces we also find intermediate cell layers. The border line between purple and white tissue is never sharp.

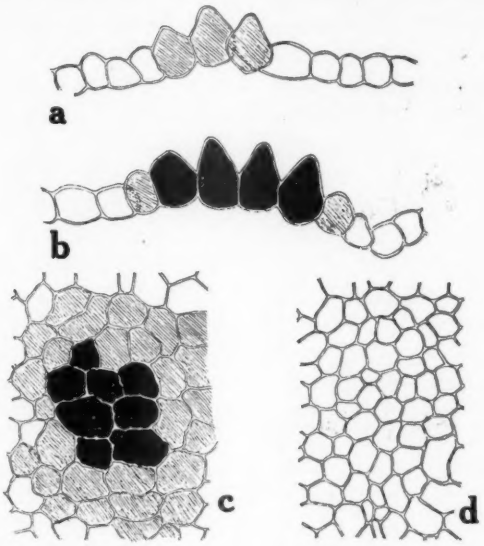

FIGURE 5.—Anatomy of a mosaic flower. a—three cells (shaded) of a light purple spot; b—four cells (represented in black) of a dark purple spot; c—a dark purple spot seen from above (dark purple cells shown in black, light purple ones are shaded); d—tissue of the white area of the petal.

Figure 6b shows a cross section of a chimeral petal. We see from this drawing that the upper epidermis of the purple area is composed of larger cells than that of the white area. The border line in this particular case is sharper than we have seen formerly between the purple spots and the surrounding white tissue. In this cross-section we see only one intermediate cell and its color is a little darker than that observed usually in the intermediate cells surrounding the dark purple spots.

DEMEREC (1931), who observed in Delphinium small purple spots surrounded by distinctly lighter cell layers, did not however find any differences in sizes between the dark purple cells in the center of the spot and the lighter purple cells of the surrounding layer. From the drawings given by this author we see that the white cells are of the same size as the dark purple and the lighter purple ones.

In Petunia the cells of the white area are much smaller than the colored cells in the center of the purple spot. The light purple cells are intermediate as to their sizes between the dark purple and the white ones.

DEMEREC assumes that the dark purple and the lighter purple cells differ genetically, namely, that the dark purple cells only have the reverted purple allele while the light purple color of the cells on the border line of purple spots is produced by some substance which diffuses from the dark purple cells into the adjacent pink cells. But the question arises whether the plant pigments can diffuse from one cell into the other. KRENKE (1933, p. 514) summarizing the results of investigations of many

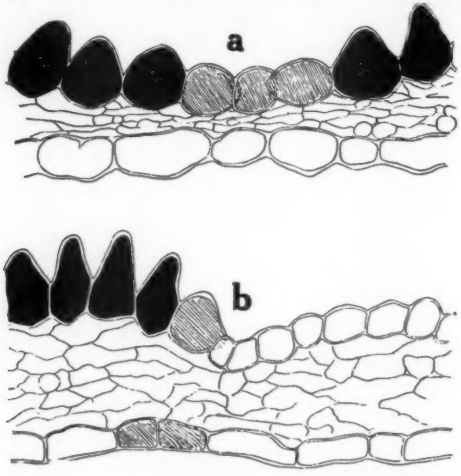

FIGURE 6.—a—cross section of the light purple cells surrounded by dark purple tissue; b—cross section of a chimeral petal of the flower shown in figure 1 G.

authors on the possibility of the passing of plant pigments from one cell into another writes as follows: "Die Farbstoffe der Pflanzen sind entweder in Protoplasma (in den Plastiden) oder aber in Zellsaft eingeschlossen. Ohne Zellverletzung (Abtötung oder Zerreissung) kommen diese Stoffe nicht einmal in die Nachbarzelle".

As I have pointed out there exists a correlation in Petunia flowers between the sizes of cells and the degree of coloration of their sap. The same causes therefore which produce lighter purple color probably determine also the smaller cell sizes.

In the smallest colored spots all cells are of a lighter purple color (the dark purple cells are missing). Such cells are also smaller than the dark purple ones characteristic of larger spots. It would be difficult to suppose that in this case the substance producing the lighter purple color of those

cells diffuses from the dark purple cells because such cells do not occur at all in the spots in question.

When two large spots are lying near each other the cells between those spots are of a lighter purple color and their sizes are a little smaller than those of the dark purple cells. Their walls are also deprived of small protuberances characteristic of the dark purple cells. Such cells are shown in cross-section in figure 6a. We see from this figure that they are smaller than the adjacent dark purple cells. Purple cells are present also on the lower surface of the petals. Here the anthocyanin pigmentation is distributed in patches or stripes running more or less parallel to the midrib of the petal. The anthocyanin distribution on the upper surface of the petal does not correspond usually to that of the lower surface.

DEMEREC assumes that each small purple spot in Delphinium is a result of mutation. "The mutations", writes this author, "occurring in the epidermal cells of sepals and petals show up as purple spots". The sizes of the spots of changed tissue, according to DEMEREC, can be used as an indication of the time when the mutation occurred, large spots being the early mutations and small spots being the late ones. I do not think we can explain in such a way the occurrence of purple spots on the petals of Petunia. Some arguments against such a supposition were quoted above. Some others will be published in another paper. It seems more probable that the mutable alleles determine different types of anthocyanin distritribution shown in figure 1. Some of those alleles may produce a few small purple spots, the others may be responsible for a larger number of large purple spots. These alleles are of pleiotropic nature and they also influence the sizes of flowers, the degree of pollen sterility, the percent of germinating seeds and so on. All these characters are strongly correlated in our unstable race of Petunia.

It is important to note that in the flowers of Petunia not only the epidermis derives from dermatogen. Dr. H. TELEŻYŃSKI found in the cytological preparations of the normal diploid race of *P. violacea* one very young flower bud showing periclinal arrangement of diploid and tetraploid tissues The tetraploid cells were found in the epidermis of this bud and also in the tissue of the petal margins, underlying the epidermis. We must assume that this tetraploid tissue originated through mutation from a diploid cell of the dermatogen layer. It would be difficult to imagine other causes which could produce in this case such a periclinal chimera. The epidermis of both the upper and the lower surface of the corolla is composed of tetraploid cells and besides the tetraploid tissue was found in the inner part of the margins of all flower lobes. I think we may safely assume that in the buds composed of diploid cells only the border line between dermatogen and periblem is the same as in those with tetrapolid dermatogen. During

the growth of flower buds the margins widen more intensively in large self-purple flowers than in small mosaic ones. Thus the differences in sizes of flowers as well as those concerning the amount of pigment are associated with the tissue derived from dermatogen.

The genes acting in the dermatogen layer produce simultaneously the definite sizes of petals and the definite amount of pigment in the epidermis. Somatic mutations occurring very often in the unstable race of Petunia and resulting in sectorial chimeras always concern both characters simultaneously. Of course the genetic constitution of the inner cell regions, de-

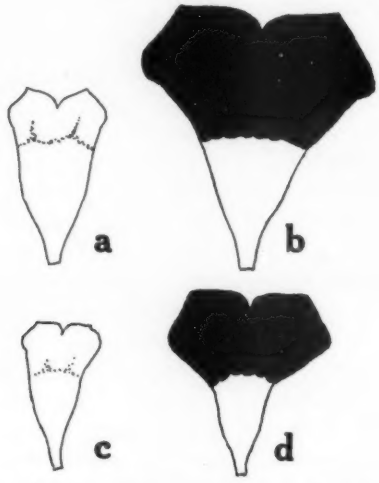

FIGURE 7.—a, b—two petal lobes and corresponding parts of the corolla tube of two flowers of a chimeral plant with large flowers (a—from a mosaic flower, b—from a self-purple flower); c, d—two petal lobes and corresponding parts of the corolla tube of two flowers of a chimeral plant with small flowers (c—from a mosaic flower, d—from a self-purple flower). Natural sizes.

rived from periblem, play also a part in the determination of flower sizes. This problem however will be discussed in another paper.

Figure 7c, d, represents the lobes and the tubes of two flowers taken from a chimeral individual. One of these flowers is self-colored (self-purple) while the other is mosaic. We see that the differences between them consist not only in the amount of pigment but also in the sizes of the lobes, the sizes of the corolla tube being the same in both flowers. The mutation which took place in this chimeral individual concerns both the sizes and the amount of pigment.

There are other chimeras in which the self-purple flowers are distinctly larger than in the above described individual. The lobes as well as the

tubes of such flowers are proportionally larger (figure 7a, b). The mosaic flowers of such chimeral plants are also larger (figure 7). It is possible that the difference between those two chimeral plants depends upon the genetical constitution of the periblem. The former chimeral individual had presumably the periblem determined by the "mosaic" allele while in the latter the periblem was possibly influenced by the "self-purple" allele. Both the smaller and the larger self-purple flowers would have on this hypothesis the same dermatogen but they would differ in the genetical constitution of the periblem. Anatomical investigations have shown that the epidermal cells of both types of self-purple flowers are of the same sizes.

THE NUMBER OF SEEDS AND THEIR GERMINATION

The plants with small mosaic flowers are smaller and weaker than those with large self-purple ones. They also have smaller fruits and a smaller number of seeds per fruit. In table 1 some data are given concerning the number of seeds per fruit in plants with large self-purple flowers and in those with small mosaic flowers.

TABLE 1

The number of seeds per fruit in two types of plants.

IN PLANTS WITH LARGE SELF-PURPLE FLOWERS			IN PLANTS WITH SMALL MOSAIC FLOWERS		
NO. OF PLANT	NUMBER OF FRUITS EXAMINED	AVERAGE NUMBER OF SEEDS PER FRUIT	NO. OF PLANTS	NUMBER OF FRUITS EXAMINED	AVERAGE NUMBER OF SEEDS PER FRUITS
A	7	171	G	9	60
B	20	143	H	24	139
C	11	149	K	16	101
D	6	260	M	13	64
F	25	127	P	12	70

The percent of germinating seeds was also greater in the plants with large self-purple flowers (table 2). I have chosen for germination tests the

TABLE 2

Seed germination.

PLANTS WITH LARGE SELF-PURPLE FLOWERS				PLANTS WITH SMALL MOSAIC FLOWERS			
NO. OF FRUIT	NUMBER OF SEEDS PER FRUIT	NUMBER OF SEEDS SOWN	NUMBER OF SEEDS GERMINATING	NO. OF FRUIT	NUMBER OF SEEDS PER FRUIT	NUMBER OF SEEDS SOWN	NUMBER OF SEEDS GERMINATING
A_1	160	144	128	H_{13}	175	144	116
A_2	195	144	134	H_{14}	175	144	112
A_3	175	144	129	H_{16}	170	144	98
A_4	150	144	123	H_{17}	175	144	105
A_6	185	144	130	H_{19}	165	144	91
A_7	148	144	122	H_{20}	160	144	100

largest fruits not differing notably as to the number of their seeds. The germination tests lasted 28 days in a temperature of 25°C during 6 hours a day and about 18°C during the remaining 18 hours.

THE POLLEN GRAINS

In the garden varieties of *P. violacea* we find usually a large percent of defective pollen grains. The same is in our unstable race. We find in this race less than 50 percent of pollen grains well formed and apparently capable of further development; the remainder are deficient in size or content and are obviously non-functional.

The two extreme flower types differ in the percent of defective pollen; this percent is larger in small mosaic flowers than in self-purple ones. We found in typical flowers of the two extreme types of plants the following numbers: in the large self-purple flowers taken at random from different pollen sacs of 4 individuals 556 well-formed pollen grains and 775 abortive and in the small mosaic flowers from 5 plants 203 well-formed and 932 abortive pollen grains. In the first case we have to deal with 58 percent of abortive pollen and the second with 82 percent. The two extreme flower types do not differ significantly as far as the percentage of germination of their well-formed pollen grains is concerned. The germination test carried out by sowing the pollen grains in 10 percent cane sugar solution has proved however that the percent of germinated pollen grains is a little larger in the normal variety of Petunia than in our unstable race. The pollen grains are also larger in the large self-purple flowers than in the small mosaic ones.

CYTOLOGICAL DATA

The experimental data so far published by the writer and by Dr. M. SACHSOWA (formerly assistant of our Institute) and concerning the unstable race of *P. violacea* seemed to favor the hypothesis of unstable genes. However in view of great differences existing between the extreme flower types and involving so many characters it was necessary, before accepting or rejecting this theory, to examine the cytological nature of the unstable race. If there were cytological differences between the extreme types of flowers it would be difficult, of course, to accept the theory of unstable genes. In 1929 I entrusted to Miss A. SMÓLSKA the task of investigating this problem. These investigations published in 1932 were inadequate because they did not take into account the morphological characters of the chromosomes. Besides, the results of these researches could not account for all characteristic features of this race. I decided therefore to examine the problem anew and this time thanks to the work of DR. H. TELEZYŃSKI results were arrived at which show that the two extreme flower types do not differ from each other as far as the morphol-

ogy of chromosomes and their behavior are concerned. Dr. TELEŻYŃSKI did not confirm the observations of Miss SMÓLSKA as to the conjugation of non-homologous chromosomes.

All individuals of the unstable race which were examined by Dr. TELEŻYŃSKI differ from the normal varieties of *Petunia violacea* in being deprived of one satellite. In normal varieties the longest chromosome pair possesses two satellites. In the unstable race one of these satellites is missing. The pair of chromosomes in question is marked in figures 8 and 9 by A. In the chromosome Ab in which the satellite is missing the terminal

FIGURE 8.—Somatic chromosome complement of the unstable race. The chromosomes which have been identified in mitosis and meiosis are marked by A and B. The chromosome Ab is without satellite. ×5200.

FIGURE 9.—Satellited chromosome pair A in somatic prophase: a—in the normal variety of Petunia violacea both satellites are present (fixed in Navashin, stained by Feulgen's method). b—in the unstable race one satellite is missing (fixed in 2BD, stained by Feulgen's method). ×4500.

part of its shorter arm is homologous with the part of the shorter arm of the chromosome B, which may be inferred from the fact of the occurrence of triple terminal chiasmata observed several times by Dr. TELEŻYŃSKI between these chromosomes (figure 10).

These observations may be interpreted in the following way: During the evolution of the unstable race of *P. violacea* structural changes in the chromosomes have occurred consisting in the interchange of the terminal parts of the short arms of the chromosomes A and B. The chromosome behavior of the plants examined shows that those plants possess two chromosomes B which are identical, one chromosome A with satellite and one chromosome Ab with an exchanged part of the chromosome B (without satellite). Thus all plants of the unstable race examined are structural heterozygotes, trisomic on account of the interchanged part of the chro-

mosome B and monosomic in consequence of the deficiency of both the satellite and the terminal part of the chromosome A. Since the structural heterozygosity occurs in all plants of the unstable race we can draw the conclusion that in this race a balanced lethal mechanism is present. The zygotes deprived of both satellites are not viable because of the deficiency of the homologous parts in two chromosomes. The lack of plants with two satellites may be accounted for by the assumption that the lethal gene is located either in the satellite or in the terminal part of the chromosome A connected with this satellite.

The course of meiosis in the unstable race is normal and it is typical for the plants with complete terminalization of chiasmata. The mean chiasma

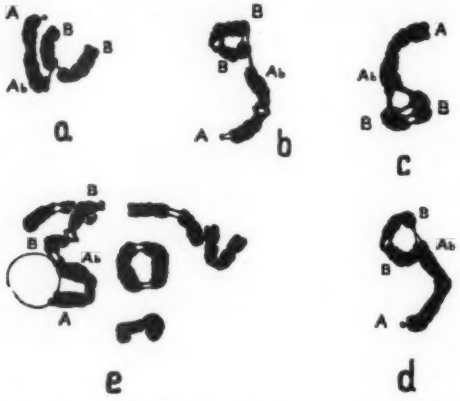

FIGURE 10.—Tetravalents in diakinesis in P.M.C. of the unstable race. In all figures the connections are seen between the chromosome Ab and one or two chromosomes of the pair B. a, b, c—tetravalents (separately represented) from different nuclei of the plant with large self-purple flowers (fixed in 2 BD, stained by Feulgen's method); d, e—tetravalents from the plant with small mosaic flowers (fixed in Bouin-Allen, stained by Feulgen's method); d—tetravalent with triple chiasma between the chromosome Ab and two chromosomes of the pair B; e—nucleus in diakinesis with 7 chromosome pairs (the tetravalent is lettered). ×4500.

frequency is approximately the same in plants of the unstable race and in those of the normal variety of *P. violacea* and the differences between particular plants are smaller than the ones between particular stamens of the same plant. The mean chiasma frequency per bivalent calculated for 1890 bivalents in 5 plants of the unstable race is 1.30. The mean number of ring bivalents per nucleus for 270 nuclei examined is 2.15, the modal value being 2. The two extreme flower types of the unstable race do not differ in this respect (table 3).

Abnormalities resulting from the association of homologous parts of the chromosomes A and B are very rare. More frequent are anomalies con-

TABLE 3

Frequency of the occurrence of ring bivalents in 270 nuclei.

FLOWER TYPE	NUMBER OF RING BIVALENTS						
	0	1	2	3	4	5	6
Small mosaic flowers	5	33	40	26	5	1	
Large self-purple flowers	5	30	63	45	13	3	1

sisting in the non-formation of chiasmata between the homologous chromosomes of one of these pairs. As a result we observe 2 univalents not associated in pairs in 2–5 percent of the pollen mother cells.

The most frequent cause of abnormalities is the phenomenon of lagging of the bivalents not completely terminalized in the anaphase I. These phenomena are apparently depending upon the external conditions, which may be inferred from the fact that the frequency of their occurrence is different in different pollen sacs of the same stamen. Such abnormalities are probably caused by the increased viscosity of the protoplasm. They occur also in normal varieties of Petunia and are probably the commonest cause of abortion of pollen grains in all varieties of *P. violacea*. The differences in the degree of pollen sterility between the two extreme flower types of the unstable race are possibly determined by the same mutable alleles which determine also the differences in sizes between the flowers. The above cytological characteristic of the unstable race is based entirely upon Dr. H. TELEŻYŃSKI'S researches carried out in our Institute. The more detailed results of these researches will be published elsewhere in a separate paper.

SUMMARY

1. The extreme types of the unstable race of *Petunia violacea* differ in the following characters: (a) in the sizes of flowers, (b) in the distribution and in the amount of pigment in flowers, (c) in the sizes of the epidermal cells of petals, (d) in the percent of abortive pollen grains, and (e) in the percent of germinating seeds.

2. In small flowers the pigment is distributed in the form of small spots. These spots increase in sizes and usually fuse with one another in larger flowers. The larger spots are composed of dark purple cells surrounded by a layer of lighter purple cells. The smallest spots are composed entirely of lighter purple cells.

3. The epidermis and the marginal cells of the petals underlying the epidermis, derive from dermatogen. This fact accounts for the correlation existing between the sizes of petals and both the amount and the distribution of pigment. The genes acting in the dermatogen layer produce simul-

taneously the definite sizes of petals and the definite amount of pigment in the epidermis.

4. In spite of great differences existing between the extreme flower types of the unstable race these extreme types do not differ cytologically.

ACKNOWLEDGMENT

I am greatly indebted to Miss E. WIŻNIEWSKA for the anatomical drawings made for this paper, and to Dr. H. TELEŻYŃSKI for lending me the figures 8, 9 and 10.

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THE EFFECT OF TEMPERATURE ON A MOTTLED-EYE[1] STOCK OF DROSOPHILA MELANOGASTER

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Received January 8, 1935

The following paper presents data on the effect of temperature on a mottled-eyed fly, a mutation appearing in a progeny of flies which had been subjected to supersonic radiations in an experiment carried out by HERSH, KARRER and LOOMIS (1930). The original stock was slightly changed by the insertion of sex-linked vermilion by W. P. SPENCER of THE COLLEGE OF WOOSTER. Such was the status of the stock at the beginning of this experiment. Mottled-eyed flies have been reported on rather numerous occasions (MULLER 1928, WEINSTEIN 1928, GLASS 1933, PATTERSON and PAINTER 1931, and GOWEN and GAY 1933). These are apparently quite different genetically.

The accompanying illustrations were prepared by Mr. EMMET MALEY, student at BALDWIN-WALLACE COLLEGE, Berea, Ohio, 1934. They more accurately reveal the nature of the mottled character than could description. These illustrations show samples of the possible variations from nearly complete black pigmentation in figure 2 (A) to a slight shadowing in figure 2 (D). Not only is there variation in the magnitude of the effect but also a considerable variation in the color and compactness of the darkened area. If only a small area is darkened, it is predominantly the dorsal posterior margin of the eye that is affected. This fact may in some way be in accord with the anterior-posterior and dorsal-ventral gradient of development.

The flies used in the experiment were carefully selected for the mottled character from the stock banana-agar bottles at WESTERN RESERVE UNIVERSITY. They had been started at 25°C primarily for increased egg production and then placed at fluctuating room temperature for development. The selected flies were placed in 6×2 bottles on banana media and held at 18°C, 20°C, 22°C, 24°C, 25°C and 29°C. The 24°C and 25°C temperatures were maintained with commercial incubators with a fluctuation of slightly less than a degree. The 18°C, 20°C, 22°C and 29°C temperatures were maintained by laboratory-constructed incubators which were controlled by toluene mercury tube thermostats. These were accurate to .5°C. An underground, unheated chamber was used in the experiment to maintain temperatures below 24°C. During the experiment all temperatures

[1] I wish to express appreciation to Professor A. H. HERSH of the Biology Department of WESTERN RESERVE UNIVERSITY for his constant and helpful advice during the course of the experimental work.

were maintained within the above limits except the 18°C temperature which fluctuated on three occasions approximately two degrees.

The families of flies containing more than fifty progeny, thus produced, were carefully examined. The flies incubated at 25°C and 29°C showed no mottling although some showed a slight suppressed shadowing of the eye as illustrated in figure 2 (D). Such flies with apparent shadowing of the eyes were not considered mottled in this experiment.

The successive increases in temperature decreased the percentage of mottled individuals. This is exemplified by the data in table 1. In addition to determining the percentage of individuals mottled the percentage of the total area of the eye affected was carefully determined. This was done by killing the flies with ether, thus causing them to extend their wings and legs. This treatment facilitated a more constant orientation of the

TABLE 1

The relation of temperature to the percentage of individuals showing the mottled effect in a population.

°C	TOTAL NUMBER EXAMINED	MOTTLED	NON-MOTTLED	PERCENT
18	487	483	4*	99.1
20	346	346	0	100
22	673	335	338	49.9
24	350	144	206	41.1
25	417	1*	416	.23
29	about 200	0	about 200	0

* The data show 4 non-mottled flies at 18° C and 1 mottled fly at 25° C. These are irregularities and may have been due to an error on the part of the author or to impurity in the line as hundreds of flies have since been produced at each of the experimental temperatures without reoccurrence.

flies in balsam. After the flies were thus carefully mounted on their left sides on microscope slides, the right eye was examined with a compound microscope. By the aid of a camera lucida, fifty eyes of fifty flies produced at each of the experimental temperatures were carefully traced on squared paper. This was made possible by the construction of a special adjustible stage lamp for the differentiation of color with the camera. From these data it was possible to determine accurately the percentage of the total

TABLE 2

The relation of temperature to the average area of the eye affected.

°C	NUMBER MEASURED	AREA (PERCENT)
18	50	83.6
20	50	77
22	50	38.1
24	50	18.6

area of the eye affected by the dark pigmentation. The main source of error in thus computing the percentage of area affected is in the orientation of the fly on the slide. Such error is probably small and does not overshadow the observable relationships. The findings are recorded in table 2.

A graphical representation of the relationship of temperature to mottling is illustrated in figure 1.

In the graph (a) represents the relation of the percentage of flies in a population showing the mottled effect to the temperature, while (b) represents the relation of the total area of the eye affected to temperature.

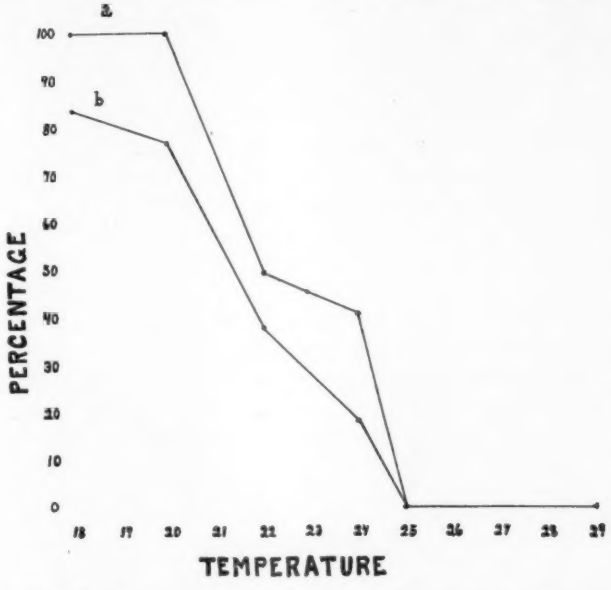

FIGURE 1.—(a). Graphic relation of temperature to the percentage mottled in a population. (b) Graphic relation of temperature to the percentage of the total area of the eye affected.

The four illustrations in figure 2 show typical mottled effects as produced (A) at 18°C, (B) at 22°C, (C) at 24°C, and (D) at 25°C. The mottled effect is distinctly variable for each of the experimental temperatures.

In the course of previous experimentation it seemed apparent that the time during development at which the mottled effect was produced was in the interval from pupation to emergence. This hypothesis was verified by the following procedure. Approximately six large families of mottled flies were started in order to produce large numbers of developing flies simultaneously. The six test-bottles were kept at 20°C. The original adults were removed at the beginning of pupation. The existing pupae

were also removed that day and once every twenty-four hours for the next successive six days. The pupae after being carefully removed from the test bottles were placed in test tubes which contained a wet piece of filter paper and which were plugged with cotton. Approximately fifty pupae were placed in each tube. The first tube of pupae was placed im-

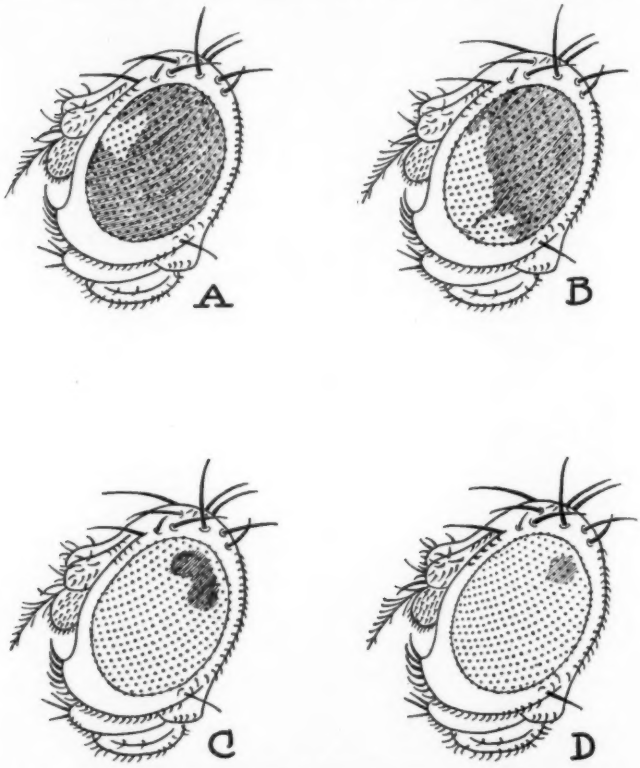

FIGURE 2.—Typically illustrates the variable appearance of the mottled eye at 18°C, 22°C, 24°C and 25°C.

mediately at 27°C and kept there until the flies emerged four days later. Each successive tube of pupae was kept at 20°C one day longer than the preceding tube and placed at 27°C until the flies emerged. As the time was lengthened at the lower temperature, the interval to the time of emergence increased. The number of flies showing the mottled effect increased until all the adult flies were mottled, and the maximum interval from pupation to emergence was ten days at 20°C. Table 3 shows the results of the various tubes of pupae.

TABLE 3

The effect of temperature change during the time interval from pupation to emergence on the mottled character.

TUBE NO.	DAYS AT 20°C	TIME TO EMERGENCE AT 27°C	NUMBER NON-MOTTLED	NUMBER MOTTLED
1	0	4 days	45	0
2	1	5	48	1
3	2	6	31	20
4	3	6	19	22
5	4	7	0	40
6	5	7	0	51
7	6	8	0	39
8	7	8–9	0	56
9	8	8–10	0	48

From table 3 it is evident that if the pupae remain at 27°C until emergence, even though pupation occurred at 20°C, the flies will be non-mottled. Thus the effective period is during the pupa stage. It is also evident from the table that if they remain approximately one-half of the time interval from pupation to emergence at 20°C, they are not affected by later temperature changes.

The previous procedure was then reversed. The larvae were allowed to pupate at 27°C and then carefully removed every twenty-four hours. The first tube of pupae was placed immediately at 20°C and successive tubes kept one, two, three, and four days at 27°C before being removed to 20°C where they were permitted to emerge. Table 4 shows the result of the versed procedure.

TABLE 4

The effect of temperature change during the time interval from pupation to emergence on the mottled character.

TUBE NO.	DAYS AT 27°C	TIME TO EMERGENCE AT 20°C	NUMBER NON-MOTTLED	NUMBER MOTTLED
1	0	8 days	0	51
2	1	6	20	17
3	2	5	57	0
4	3	5	54	0
5	4	4	22	0

Thus if the pupae remain at 20°C until emergence, even though pupation occurred at 27°C, the flies will be mottled. It is again evident that if they remain approximately one-half of the time interval during the pupa stage at 27°C, they are not affected by later temperature changes. It may thus be said that the effective period for mottled-eye is during the first half of the time interval from pupation to emergence.

SUMMARY

1. The mottled-eye character is affected by temperature.

2. The percentage of individuals showing the effect in a population increases with a decrease of temperature over a range of 27°C to 18°C.

3. The area of the eye affected increases with a decrease of temperature over a range of 24°C to 18°C.

4. The mottled effect is erased at incubation temperatures of 24°C or above.

5. The effective period is during the first half of the time interval from pupation to emergence.

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THE LACK OF EFFECT OF ETHERIZATION ON THE X-RAY MUTATION RATE IN DROSOPHILA SIMULANS

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Received January 10, 1935

The present investigation arose out of a more general study which the author undertook concerning the production of mutations in *Drosophila simulans* as compared with that in *Drosophila melanogaster* (KOSSIKOV 1934 in press). Since for this study it was necessary to conduct X-ray mutation experiments, it seemed advantageous to increase the significance of the work by varying, in certain series, the conditions under which the treatments were given. Anesthesia by ether was decided upon as a convenient influence to use in this connection, and also one of some practical importance in the Drosophila work, since the degree of anesthetization in ordinary X-ray mutation experiments in flies may be subject to considerable variation when no particular attention is paid to this factor, and hence it is important to know whether this may constitute a source of variation in the results. According to earlier work of HANSON and HEYS on *Drosophila melanogaster*, there did seem to be some such effect, but since we were here able to obtain further evidence on this subject, derived from a different species, it was thought of greater interest to strengthen and extend our knowledge of the phenomenon in question than to reach out in a new direction before the earlier work had had a chance to become well grounded.

MATERIAL AND METHODS

Wild-type males of *Drosophila simulans* (from the so-called "St. Augustine" strain) were subjected to X-rays. A part of these males were left in full narcosis during the X-ray treatment, in a special glass apparatus constructed for this purpose, while the rest, designated as "controls", were not etherized but were X-rayed simultaneously, with the same dosage of X-rays, all other conditions being identical. A heavier and a lighter dose of X-rays was used, in different experiments, the lighter being about 1,100 and the heavier about 2,700 r, judging by the lethal rate in parallel *ClB* tests on *Drosophila melanogaster*. Imperfections in the dosimeter measurements resulted in readings about three times the actual value, but other work done at our laboratory, with another dosimeter, has agreed with previous work in showing a constant relation between the lethal rate given by the *ClB* method, and the actual r units, so that the former may be taken as providing a reliable method of calibration. The X-rayed males just after treatment were crossed to virgin females homozygous for the genes yellow, dusky, forked. In the second generation, recessive sex-linked

lethal mutations were registered, which could be found by the absence of non-crossover males of the wild-type in the individual cultures.

The precise registration of recessive lethal mutations in the X chromosome of *Drosophila simulans* offers some difficulties, since we have not yet got a method like the *ClB* method in this species of Drosophila. In order to count all the lethal mutations more exactly, however, an inversion in the X chromosome was used, which had been obtained during the preliminary part of this work, undertaken for this purpose. This inversion considerably reduces the frequency of crossing over in the region of the dusky gene.

All the cases in which there was doubt concerning the presence of a lethal were analyzed in the third generation. The flies were reared at a temperature of 25°-26°C on a yeast-containing medium, the formula of which had been worked out by C. A. OFFERMANN (See Drosophila Information Service No. 3).

RESULTS OBTAINED AND DISCUSSION

Among 1,480 F_2 cultures obtained from males which were X-rayed under narcosis with an estimated dose of 1,100 r, 50 lethals were registered; this constitutes 3.4 ± 0.5 percent (the error here given being the standard error). On the other hand, out of 1,554 F_2 cultures of the control series, 47 lethals were obtained, that is, 3.0 ± 0.4 percent.

In the second series of experiments, in which the X-ray treatment was increased up to an estimated dosage of 2,700 r, 41 lethals were registered out of 384 cultures, or 10.7 ± 1.6 percent, in the narcotic series, and 56 lethals out of 479 cultures, or 11.7 ± 1.5 percent, in the control series (see table 1).

TABLE 1

SERIES	DOSAGE (ESTIMATED)	ETHER+X-RAYS				X-RAYS ONLY			
		NO. OF F_2 CULTURES	NO. OF LETHALS AND SEMI-LETHALS	PERCENT (WITH ITS STANDARD ERROR)	VISIBLE MUTATIONS	NO. OF F_2 CULTURES	NO. OF LETHALS AND SEMI-LETHALS	PERCENT (WITH ITS STANDARD ERROR)	VISIBLE MUTATIONS
1	1100r	334	15	4.5 ± 1.2	..	329	15	4.6 ± 1.2	..
2	1100r	361	16	4.4 ± 1.1	..	350	12+1	3.7 ± 1.0	..
3	1100r	785	17+2	2.4 ± 0.6	..	875	16+3	2.2 ± 0.5	1
Sum 1–3	1100r	1480	48+2	3.4 ± 0.5	..	1554	43+4	3.0 ± 0.4	1
4	2700r	384	37+4	10.7 ± 1.6	1	479	49+7	11.7 ± 1.5	3+1(?)

These figures clearly show that there is no difference in the genetic effectiveness of X-ray treatment, when it is applied to non-narcotized flies and to flies that are kept under narcosis throughout the treatment.

It will be seen that the present results failed to confirm the earlier conclusions concerning the effect of etherization on X-ray mutation frequency

in *Drosophila melanogaster*, referred to in the introduction. They agree, however, with the recent results of HANSON, investigating the same question in *Drosophila melanogaster* in a series of parallel experiments which are being published simultaneously (in press, 1934). This shows that there is no essential difference between *Drosophila simulans* and *D. melanogaster* in this respect, and the mutually confirmatory nature of our parallel sets of observations leaves no doubt about the essential correctness of the present conclusion, that is, if there is an effect of etherization, it must be a very small one, and need not ordinarily be taken into account since in the present experiments the flies were under deeper narcosis during treatment than they ever are in ordinary X-ray experiments.

In this connection it is of interest also to call to mind certain unpublished experiments of GERSHENSON, in which ether alone, without X-raying, was used in the attempt to produce mutations (the *ClB* method of detection being employed) and no significant rise in mutation frequency was found. Still earlier, MORGAN (1914) had reported on the failure of ether to produce mutations, but at that time the methods of detection were not delicate enough to have given a positive result, even if the mutation frequency had been raised many times. It should be noted, moreover, as a matter of principle, that it is quite possible for an agent in itself to affect the mutation rate and still to have little or no influence in changing the mutation rate caused by another simultaneous agent, such as X-rays; in fact, the results found in the case of temperature with and without X-rays by MULLER (1928,1930) and by TIMOFEEFF-RESSOVSKY (1934) are a case in point.

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THE Y CHROMOSOME OF DROSOPHILA PSEUDOOBSCURA

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Received January 10, 1935

INTRODUCTION

The variation in the shape of the Y chromosome of *Drosophila pseudoobscura* Frolowa was first observed by LANCEFIELD (1929). He found that race A of this species has a J-shaped, and race B a V-shaped Y chromosome. This difference in the shape of the Y chromosome remains the only known "morphological" differential between the two races of this species, which are otherwise distinguished by their behavior in crosses (LANCEFIELD, l. c.), and by a number of physiological characteristics (POULSON 1934, DOBZHANSKY 1935, BOCHE unpublished). DOBZHANSKY and BOCHE (1933) observed that the shape of the Y chromosome does not remain constant even within a race; two types of Y chromosomes occur in race A, and two other types in race B. Further studies, to be reported in the present paper, have increased the number of the types of Y chromosomes known in the species up to at least six, and shown that each of these types occurs in populations inhabiting a distinct geographic area.

MATERIAL AND METHODS

Wild strains of *Drosophila pseudoobscura* were collected, as far as possible, throughout the specific range. Flies were captured in traps containing fermenting banana mush; traps were exposed on trees and bushes, preferably far from human habitations (because of the possibility that flies are transported from place to place by man). Flies come into the traps, frequently in great numbers, especially during the evening hours. Usually more than one strain was isolated from each of the populations thus obtained. Each strain was derived from a single female fertilized in nature by one or more males. The strains were designated by the names of the localities in which their wild ancestors had been collected, and by serial numbers. Thus, "Seattle-6" is the strain No. 6 from Seattle, Washington.

The writer wishes to acknowledge his obligations to Miss C. V. BEERS, Miss M. GROSCURTH and Messrs. R. D. BOCHE, V. M. EMMEL, M. M. LERNER, D. F. POULSON, C. N. RUDKIN, A. H. STURTEVANT, and R. C. WARNER who collected a part of the material.

The shape of the Y chromosome was determined by examining the spermatogonial metaphase plates. In *Drosophila pseudoobscura* the spermatogonial divisions are numerous enough in the testes of the adult males to permit a determination of the chromosome group in practically every specimen. Most of the material was studied in aceto-carmine smear

preparations, and the remainder in sections fixed in Flemming's or Benda's fluids and stained with iron haematoxylin. Five or more chromosome plates were examined before the kind of the Y chromosome present in a given strain was considered established. This was necessary because the differences between some of the types of Y chromosome (types I and III, and types IV and V, see below) are relatively small.

The cytological examination was performed as a rule several fly generations after the strains were brought to the laboratory. In no case was more than one type of the Y chromosome observed in a single strain (this possibility could not be excluded *a priori*, since some of the original females might have been fertilized in nature by several males possessing Y chromosomes of different types).

CYTOLOGICAL CHARACTERISTICS OF THE TYPES OF Y CHROMOSOME

The Y chromosome of *Drosophila pseudoobscura* may have a median, submedian, or subterminal spindle fibre attachment. The two limbs of the chromosome vary in their absolute and relative lengths; these variations permit the identification of the following six types of Y chromosomes (a number of figures showing the types I, II, IV and V have been published in DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1934 and DARLINGTON 1934. In DOBZHANSKY and BOCHE's paper type IV was referred to as the "third type", and type V—"the fourth type").

Type I (figures 1 and 2). The Y chromosome is V-shaped, the spindle attachment is submedian. The length of the long limb of the Y is approximately equal to that of either limb of the X chromosome. The ratio of the lengths of the two limbs of the Y is about 6:4 or 6:4½. The long limb frequently shows a constriction or a bend somewhat distally from the middle of its length.

Type II (figures 3–6). A V-shaped chromosome with a more nearly median spindle attachment than in type I. The ratio of the two limbs is 3:4 or 3½:4. The longer limb is clearly shorter than either limb of the X. No constrictions are visible in either arm. Type II can be derived from type I by a loss of the distal portion of the long limb of the type I, from the constriction to the end. On this supposition, the longer limb in type II is homologous to the shorter limb in type I, and the shorter limb in type II is a homolog of a part of the longer limb in type I.

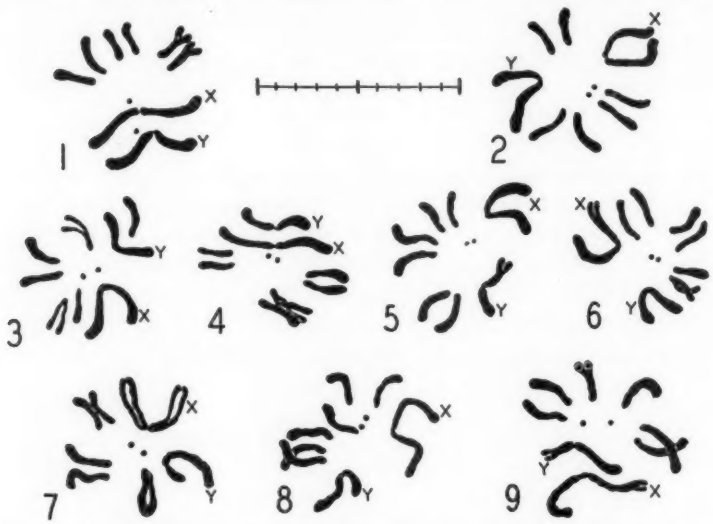
Type III (figures 7–9). A markedly unequal armed, V-shaped chromosome. The ratio of the two limbs is approximately 6:3 or 6:2½. This type can be derived from type I by a loss of a portion of the shorter limb.

Type IV (figures 10 and 11). A J-shaped chromosome with a subterminal spindle attachment. The length of the longer limb approaches that of either limb of the X chromosome, and is greater than that of the

longest autosome (figure 11 is rather exceptional in this respect). The longer limb sometimes shows a constriction at about the middle of its length.

Type V (figures 12–14). This type can be derived from the type IV by a loss of a portion of the longer limb. The longer limb in type V is about as long as the shortest rod-shaped autosome.

Type VI (figures 15–20). A relatively very short chromosome with a nearly median spindle attachment. The length of either arm is clearly

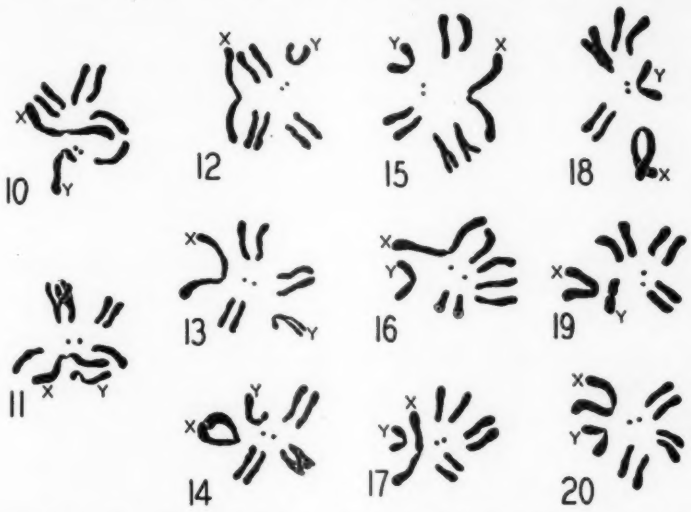


FIGURES 1–9.—Chromosomes of race B of *Drosophila pseudoobscura*. Figures 1 and 2—type I (Lassen-7); Figures 3–6—type II (Seattle-4); Figures 7–9—type III (Sequoia-5). The scale (10μ) is common to figures 1–20.

smaller than that of the shortest rod-shaped autosome. The derivation of this type is not quite clear. It might conceivably arise from type V by an inversion transposing the spindle attachment from the subterminal to the median position.

The strains of *Drosophila pseudoobscura* are classified as to race on the basis of genetic tests: the cross B ♀ × A ♂ gives sterile F_1 males having testes much smaller than normal in size, and the F_1 hybrid males from the cross A ♀ × B ♂ are sterile but have normal sized testes (LANCEFIELD 1929). Such tests were performed on all the strains in which the shape of the Y chromosome was determined. The results show that Y chromosomes of the types IV, V and VI occur exclusively in males of A race. Similarly, the types I and II are found in race B only. Type III was observed in several

B race strains, and in a single A race strain (Sequoia Park-7). The latter case deserves some consideration, since it is difficult to see how a Y chromosome of race B could be transferred into race A, or *vice versa*. Three possibilities can be suggested: (1) type III is the ancestral type of Y chromosome that has been retained only in some race B strains and in a single known race A strain; (2) non-disjunction of the sex chromosomes in an interracial hybrid resulted in an appearance of an XXY female, and a fertile male carrying the X chromosome of one race and the Y of the other emerged in its offspring; (3) the Y chromosome of the Sequoia-7



FIGURES 10–20.—Chromosomes of race A. Figures 10 and 11—type IV (Merritt-2); Figures 12–14—type V (The Dalles-5); Figures 15–20—type VI (Cedar City).

strain belongs to a separate type which is merely superficially similar to the type III. The third possibility seems most plausible. If this were established, the number of the known types of Y chromosomes in the species *Drosophila pseudoobscura* would be actually seven rather than six.

GEOGRAPHIC DISTRIBUTION OF THE DIFFERENT TYPES OF Y CHROMOSOME

The strains that were examined for the type of Y chromosome are listed below. The number of chromosome plates observed for this purpose is given in brackets after the name of each strain. Thus, "Vancouver-1 (10)" indicates that ten metaphase plates have been inspected in the strain No. 1 from Vancouver, British Columbia.

Type I (Race B)

Vancouver Island: Cowichan Lake-2 (7).

British Columbia: Vancouver-3 (5), Yale-5 (5), Yale-6 (5), Merritt-3 (11), Merritt-4 (3), 150-mile House-5 (5), Quesnel-5 (5).

Washington: Cape Flattery-8 (11), Olympic Mts.-3 (8), Olympic Mts.-5 (7), Olympic Mts.-6 (8), Seattle-2 (12), Seattle-6 (21), LaGrande-4 (10), Chehalis-3 (7).

Oregon: Newport-5 (18), Reedsport-3 (7), Days Creek-2 (6), Crater Lake-4 (8), Sisters-9 (9).

California: Klamath-5 (6) Shelter Cove-1 (3), Shelter Cove-6 (7), Mendocino-1 (18), Dunsmuir-1 (10), Dunsmuir-2 (9), Lassen-4 (5), Lassen-7 (5), Mammoth Lake-1 (9), Sequoia Park-10 (9), Sequoia-8 (18), Greenhorn Mts.-6 (9), Greenhorn Mts.-8 (7), Santa Lucia-11 (9).

Type II (Race B)

Vancouver Island: Campbell River-1 (8), Campbell River-3 (10), Campbell River-4 (9), Cowichan Lake-6 (4).

British Columbia: Vancouver-1 (10), Yale-3 (9).

Washington: Cape Flattery-1 (10), Cape Flattery-3 (6), Cape Flattery-7 (5), Olympic Mts.-4 (9), Seattle-4 (23).

Type III (Race B)

California: Mammoth Lake-10 (12), Sequoia Park-4 (7), Sequoia Park-5 (6), Greenhorn Mts.-3 (10), Kern Canyon-7 (6), Santa Lucia-6 (7), Nojogui-3 (8), Nojogui-8 (8)

Type III (?) (Race A)

California: Sequoia Park-7 (18).

Type IV (Race A)

British Columbia: Yale-5 (7), Pavilion-3 (12), Pavilion-5 (8), Kamloops-2 (9), Merritt-2 (5), Lake Okanagan-1 (9).

Washington: Olympic Mts.-2 (14), Seattle-1 (9), Seattle-5 (14), LaGrande-2 (14), Chehalis-6 (5), Lake Chelan-1 (6), Lake Chelan-2 (5), NE of The Dalles-6 (8).

Oregon: Sisters-4 (7), Paulina Lake-3 (9), Crater Lake-3 (14).

California: Dunsmuir-4 (8), Lassens-1 (6), Mammoth Lake-2 (5), Mammoth Lake-8 (F 10), Greenhorn Mts.-2 (5), Greenhorn-4 (6), Greenhorn-5 (5), Greenhorn-10 (5), Greenhorn-11 (5), Greenhorn-12 (5), Kern Canyon-1 (6), Kern-2 (5), Kern-3 (5), Kern-4 (8), Kern-5 (5), Santa Lucia-1 (4), Santa Lucia-2 (6), Santa Lucia-5 (5), Santa Lucia-8 (5), Santa Lucia-10 (8), Nojogui-2 (7), Nojogui-4 (5), Nojogui-7 (6), Pasadena-1 (4), Pasadena-2 (7), Pasadena-3 (5), Pasadena-4 (5), San Bernardino Mts.-3 (5), Henshaw Lake-1 (17).

Type V (Race A)

British Columbia: Lake Okangan-2 (8), Shuswap Lake-1 (5), Shuswap Lake-3 (9), Arrowhead-5 (5), Nakusp-2 (9), Nakusp-3 (9), Kaslo-3 (13).
Washington: The Dalles-5 (5), Metaline Falls-4 (8).
Idaho: Lake Coeur d'Alene-6 (5), Idaho Falls (10).
Montana: Bitterroot Mts.-1 (6), Bitterroot Mts.-2 (9).

FIGURE 21.—The known geographic distribution of the types of the Y chromosome.

Colorado: Aspen-1 (11), Aspen-2 (6).
Utah: Bryce (9), Zion-1 (10).
Texas: Georgetown (13).

Type VI (Race A)

Utah: Cedar City (24).

The above data are summarized in figure 21 in map form. It may be regarded as established that each type of Y chromosome has a definite

geographic distribution, albeit in a number of localities more than one type is encountered in the population. Type I is found almost everywhere within the area of race B. Type II is restricted to the region around Puget Sound. Type III is endemic in the Sierra Nevada and the southern Coast Ranges. Western representatives or race A have the Y chromosome of type IV, and eastern ones of type V. Types IV and V are encountered together only in a relatively narrow stretch in British Columbia and on the lower Columbia River. Type VI is known thus far from a single locality in southern Utah. Since, however, the characteristics of the population inhabiting Arizona and New Mexico are as yet unknown (the species *pseudoobscura* does occur at least in Arizona, and probably in New Mexico and in northern Mexico), the distribution of type VI may be much wider that it appears at present.

INDEPENDENCE OF THE GENETIC CHARACTERISTICS FROM THE TYPE OF THE Y CHROMOSOME

A number of attempts were made to find a correlation between the type of the Y chromosome present in a given strain and some other properties of that strain. Thus far these attempts have been unsuccessful

Hybrids between strains of the same race having different types of Y chromosome are fertile in both sexes. The F_1 hybrid males from the interracial crosses are, of course, sterile, but there exist valid reasons for doubting whether this sterility has anything to do with the Y chromosome (DOBZHANSKY 1933). Originally it was deemed probable that the type of the Y chromosome determines, at least partly, the "strength" of a given strain. The "strength" of a strain is measured by the degree of the disturbance of the spermatogenesis observed in the hybrids between that strain and a standard strain of the other race (DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1934). The information now available shows this correlation to be spurious. Indeed, the strains "Texas" and "Shuswap-3" have the same type of Y chromosome, but the former is the strongest and the latter is the weakest A race strain known. Shuswap-3 and Chelan-2 are both very weak A race strains, but they have different types of Y chromosomes. Similar information is available for race B. As shown above, each type of Y chromosome occurs in a definite geographic region; variations in strength likewise involve a geographic element (unpublished data). These variables are, however, independent. The unpublished data of STURTEVANT on the geographic distribution of autosomal inverted sections and of the so-called sex-ratio factors also indicate no correlation with the type of the Y chromosome.

The lack of influence of the type of the Y chromosome on the genetic properties of the strain carrying it is not altogether unexpected. The Y

chromosomes of *Drosophila melanogaster* and *simulans* are known to be practically devoid of genetic factors. It is almost certain that the Y of *pseudoobscura* is likewise inert (the XO males are not visibly abnormal though they are sterile, and the Y chromosome is invisible in the cells of the larval salivary glands). It is just this peculiarity of the Y as compared with the rest of the chromosomes that makes the former especially liable to undergo far reaching alterations without endangering the life of the organism. It should be pointed out in this connection that racial variability of the Y chromosome within a species is observed not only in *Drosophila pseudoobscura* but also in *Drosophila simulans* (STURTEVANT 1929 and HEITZ 1933) and in *Drosophila caribbea* (KAUFMANN, personal communication). The well-known results obtained by STERN in *Drosophila melanogaster* are also a case in point.

Our inability to detect any influence of the variations in the Y chromosome on the morphological or physiological characteristics of its carrier does not constitute a conclusive proof of the non-existence of such influences. Nothing is known about the mechanism of the origin of the racial variations in the Y chromosome, nor do we know anything about the way these variations become established in nature. A number of interesting problems confront us in this field.

DISCUSSION

In accordance with the data presented above, the species *Drosophila pseudoobscura* might be subdivided into at least six races having different shapes of the Y chromosome and different geographic distributions. A review of the literature, even excluding cases of polyploidy and of abnormalities observed in single individuals, shows that chromosomal variations within a species are not uncommon. The first thoroughly established instance of this nature is that of *Galtonia candicans* (S. NAVASHIN 1912, 1927). Two kinds of individuals are found in this plant: some have two large satellites on a certain pair of chromosomes, and others have one chromosome with a large and one with a small satellite. The biotype having two small satellites is inviable.

Races having different chromosome numbers are known in the moth *Phragmatobia fuliginosa* (SEILER 1917, 1925), the Orthopteron *Gryllotalpa vulgaris* (PAYNE 1916, VOINOV 1925, DE WINIWARTER 1927), in *Viola kitaibeliana* (CLAUSEN 1927), in flax, *Linum usitatissimum* (EMME and SCHEPELJEVA 1927), in *Ranunculus acris* (SOROKIN 1927), and in *Scirpus palustris* (HAKANSSON 1929). The classical example of variations in the chromosome number within a species described by WILSON (1909) in Metapodius seems to belong to a different category from the above, and is probably related to cases of the rather mysterious "extra chromosomes"

found more recently by KUWADA, RANDOLPH and MCCLINTOCK in *Zea mays* (AVDULOV 1933), by GOTOH in rye (LEWITSKY, MELNIKOV and TITOVA 1932), and by AVDULOV and TITOVA (1933) in *Paspalum stoloniferum*. The "extra chromosomes" represent either multiplications of one of the chromosomes of the normal set (Y chromosome in Metapodius), or, as in cases of Zea and rye, they are elements finding no homologs among the normal chromosomes, and appear to be composed of inert or semi-inert material. The chromosomal variations observed by SEILER (1922a, b) in *Solenobia pineti* and *triquetrella*, by DEDERER (1928) in *Philosamia cynthia*, and by THOMSEN (1927) in *Aleurodes proletella* may belong to either category.

Racial differences in the visible chromosome morphology have been established in Galtonia (NAVASHIN, l.c.), in some species of grasshoppers (CAROTHERS 1917, HELWIG 1929, 1933, KING 1923), in *Beta vulgaris* (KUZMINA 1927), and in *Vicia angustifolia* (SWESCHNIKOWA 1928, 1929). Amongst the examples just quoted those of Phragmatobia, Gryllotalpa, and the grasshoppers are especially interesting since these "chromosomal races" seem to have definite geographic distributions.

Differences in the internal structure of the chromosomes are no less important than those in the visible morphology or in chromosome numbers. Rearrangements of the "normal" order of genes within the chromosomes are known to occur in nature in Datura (BLAKESLEE 1929) and in *Drosophila melanogaster, simulans* and *pseudoobscura* (STURTEVANT 1931, LANCEFIELD 1929, unpublished data of STURTEVANT and TAN). Ring configurations observed at meiosis in interracial hybrids indicate that the parental races differ in having translocations. Such configurations are known in Oenothera, Datura, Pisum, Tradescantia, Rosa, Campanula, Aucuba, and other plants. Configurations described by HEBERER in races of the Copepod *Diaptomus castor* (1924) are best interpreted as being due to translocations. Differences in the chromosome numbers (except polyploidy) and in the visible chromosome morphology imply, in fact, the existence of internal reconstructions in the chromosomal apparatus.

All these data indicate that the process of race and species formation may be resolved into at least two components: the genic differentiation and the differentiation in the chromosome structure. These two components seem virtually independent, but the phenomena of position effects may lead us to a recognition of an ultimate connection between the two. It should, however, be emphasized that the differences between races and species may be in some cases predominantly genic, and in others predominantly chromosomal in nature. The probable dual nature of the phenomenon of hybrid sterility (DOBZHANSKY 1934) indicates that, in its generalized form, the problem of race and species formation is reduced to

that of the origin of dynamic discontinuities of the living matter, rather than to that of the origin of one or the other class of specific differences.

SUMMARY

1. The Y chromosome of *Drosophila pseudoobscura* may have a median, submedian, or subterminal spindle fibre attachment. The absolute and relative lengths of the two limbs of the chromosome vary, giving rise to six cytologically recognizable types of the Y chromosome.

2. Three of the six types are encountered only in race A of the species, two others only in race B, and one type is found in several B race strains and in a single A race strain from the same locality.

3. Each of the six types occurs in wild populations inhabiting a definite geographic area (figure 21).

4. The morphological and physiological properties of a given strain are apparently not affected by the type of the Y chromosome present in that strain.

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DROSOPHILA MIRANDA, A NEW SPECIES

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Received January 30, 1935

INTRODUCTION

The species of Drosophila to be described in the present paper has certain properties that make it particularly interesting from the point of view of genetics. This species, to be designated *Drosophila miranda* Dobzhansky, is related to, and can be crossed with, a species previously known and rather extensively used for genetic purposes, namely *Drosophila pseudoobscura* Frolowa. The two species are so similar in appearance that most taxonomists would hesitate to separate them on the basis of the morphological differences alone. They represent, however, two different reaction systems, as shown by the presence of some clear-cut physiological differentials. Furthermore, the structure of the chromosome apparatus in *Drosophila miranda* is unique among the known species of Drosophila, and is, in fact, rare elsewhere. Sex determination in *Drosophila miranda* is complicated by the presence of three instead of two sex-determining chromosomes, and is based on a precise functioning of a singular mechanism that insures the proper distribution of the sex-determining chromosomes at the meiotic divisions.

Certain morphological differences between *miranda* and *pseudoobscura* were first noticed by Dr. A. H. STURTEVANT, who has also contributed a number of very valuable suggestions and criticisms; to him the writer wishes to express his sincerest appreciation.

MATERIAL

Several strains presumed to be *Drosophila pseudoobscura* were brought by Mr. R. D. BOCHE from Seattle, Washington in 1932. Each strain was derived from a single fertilized female caught outdoors. In one strain, designated "Seattle-7," the flies were much larger than in others. The Seattle-7 strain died off before any tests could be made. In 1934 the writer collected a number of strains in the same geographic region. Among these, three strains produced very large flies. The three strains are Cowichan-1, Cowichan-7 (derived from single females collected at Cowichan Lake, Vancouver Island), and Olympic-1 (collected in the Olympic Mountains, near Brinnon, Washington). These strains proved to belong to *Drosophila miranda*, a species different from *Drosophila pseudoobscura*.

Drosophila miranda was not encountered in various localities where collecting was made in Alaska, eastern British Columbia, and the western United States. Its geographic distribution is probably restricted to the region around Puget Sound.

MORPHOLOGY

Drosophila miranda is similar to *pseudoobscura* in most external characteristics used for distinguishing species of Drosophila (STURTEVANT 1921). The differences observed are summarized in the following description.

Drosophila miranda, sp. nov.

Differs from *Drosophila pseudoobscura* Frolowa by a larger size (living females of *miranda* developed at 24½°C are about 3.1 mm long, males 2.8 mm, *pseudoobscura* females 2.7 mm, males 2.5 mm), a darker coloration of the whole body, less distinct grayish stripes on the thorax, and slightly wider cheeks in relation to the longer diameter of the eye (the ratio being about 1:3 in *miranda*, and about 1:4 in *pseudoobscura*). Both species have eight rows of acrostichals between the dorsocentral bristles, two sex combs on the anterior tarsi in the males, and two egg filaments; the chaetotaxy, the facial carina, the number of branches in the arista, and the genitalia of both species (studied in a material cleared in a KOH solution and glycerine) are alike. The type is in the collection of the AMERICAN MUSEUM OF NATURAL HISTORY.

Accurate measurements of the length of the body are difficult in Drosophila on account of the telescopic movements of the abdomen and the looseness of the joints between the head and the thorax and the thorax and the abdomen. The length of the hind tibiae was therefore measured with the aid of an ocular micrometer (in cleared flies). The results are summarized in table 1. The differences between *miranda* and *pseudoobscura* are clearly statistically significant.

TABLE 1

Length of the hind tibiae in (*at 24½°*).

SPECIES	RACE	STRAIN	MALES				FEMALES			
			M±m	σ	LIM	n	M±m	σ	LIM	n
pseudoobscura	A	Okanagan-3	715		661–748	43	746±2.9	16.2	714–782	32
	A	Olympic-2	707		678–748	24				
	A	Average	712±2.8	22.6	661–748	67	746±2.9	16.2	714–782	32
	B	Cowichan-2	644		609–678	19				
	B	Shelter Cove-1	663		626–695	39				
	B	Average	656±2.9	21.9	609–695	58				
miranda		Olympic-1	771		713–818	41				
		Cowichan-1	779		713–818	60				
		Cowichan-7	777		731–818	35	831±2.4	15.3	800–870	37
		Average	777±2.1	25.2	713–818	136	831±2.4	15.3	800–870	37

Another difference between *miranda* and *pseudoobscura* involves the number of teeth in the sex combs (table 2).[1] *Miranda* has larger sex combs than *pseudoobscura*. The proximal sex comb (located on the first tarsal joint) is considerably larger in *miranda* than the distal comb (on the second tarsal joint).

TABLE 2

Number of teeth in the sex combs (at 24½°).

SPECIES	RACE	STRAIN	DISTAL COMB				PROXIMAL COMB			
			M±m	σ	LIM	n	M±m	σ	LIM	n
pseudoobscura	A	Okanagan-3	5.14		4–7	43	6.60		6–8	43
	A	Sequoia-6	5.35		4–7	46	6.89		6–9	46
	A	Chehalis-5	5.08		4–6	25	6.48		6–8	25
	A	Portland-2	5.04		4–6	26	6.19		6–7	26
	A	Olympic-2	5.46		4–7	25	6.42		6–8	25
	A	Average	5.22±.05	.60	4–7	165	6.57±.05	.65	6–9	165
	B	Cowichan-2	4.58		4–5	19	5.84		5–6	19
	B	Klamath-5	4.83		4–6	29	5.93		5–7	29
	B	Shelter Cove-1	4.90		4–6	40	5.63		5–6	40
	B	Average	4.81±.06	.60	4–6	88	5.77±.06	.54	5–7	88
miranda		Olympic-1	5.81		5–8	41	8.71		7–10	41
		Cowichan-1	5.73		5–7	60	8.33		7–10	60
		Cowichan-7	5.90		5–7	31	8.29		6–10	31
		Average	5.80±.06	.68	5–8	132	8.44±.07	.81	6–10	132

There is no difficulty in distinguishing *Drosophila miranda* from *pseudoobscura* by body size, provided both species have developed under similar environmental conditions. However, body size in Drosophila is rather strongly affected by temperature and food conditions (ALPATOV 1930), and *miranda* developed at 25° in crowded cultures is not sufficiently different from *pseudoobscura* developed at 15° in cultures well provided with food to permit a clear separation. In fact, the original *miranda* females caught in nature struck the eye by their large size, but at least one other female that seemed to be as large as they were produced offspring that proved to be race B of *pseudoobscura*.

[1] Data presented in tables 1 and 2 suggest the existence of a morphological difference between race A and race B of *pseudoobscura*. Up to now no such difference has been recorded. It remains to be ascertained whether this difference persists at all temperatures. Temperature of 24½° is less advantageous for race B than for A, and the smaller size of race B may be merely an expression of the unfavorable effects of heat on race B. KOLLER (1934) has stated that race A has longer and narrower testes than race B. I can not confirm this observation.

PHYSIOLOGICAL CHARACTERS

According to POULSON (1934) the development of *Drosophila pseudoobscura* takes, at 25°, thirteen to fourteen days (from oviposition to the emergence of the adult), about ten hours more in males than in females, and about ten hours more in race B than in race A. The development of *miranda* females takes, at the same temperature, about fifteen days, and males are very much slower than females. If parents are allowed to oviposit for only 24 hours, the emergence of the adult female offspring ends before the emergence of the males begins. For a day, or even two, no flies at all emerge in the cultures, and this period is followed by three or four days during which the males emerge. If the parents are allowed to oviposit for a longer time in the same culture, the times of the emergence of the males and females naturally overlap, but it remains apparent that the sexual dimorphism in the time of development is stronger in *miranda* than in any other species of Drosophila with which the writer is familiar.

Newly emerged females have undeveloped ovaries in both *miranda* and *pseudoobscura*, but in the former the full sexual maturity is reached at least two days later than in the latter (at room t°, about 22°). *Miranda* is more sensitive to high temperatures than *pseudoobscura;* at 25° *miranda* females are mostly sterile. At temperatures around 20° both species, however, seem to be about equally vigorous.

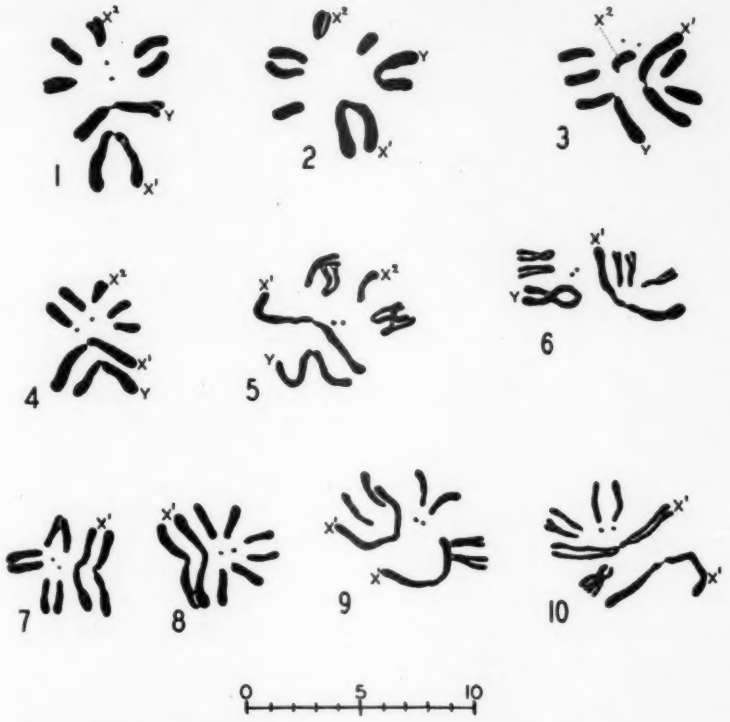
CHROMOSOMES

Chromosomes of *Drosophila miranda* were examined in the spermatogonial divisions (figures 1–4), in the nerve cells of the larval ganglia (figures 5, 6, 9, 10), and in the oogonia (figures 7 and 8). Spermatogonial divisions were seen in all three strains available (Olympic-1 Cowichan-1, Cowichan-7). The aceto-carmine smear method was used in most cases, but sections of the testes were also studied. As a supplement, the chromosomes were investigated also in the larval salivary glands, following the technique of PAINTER (1934).

Drosophila miranda females have a chromosome group resembling that of *Drosophila pseudoobscura* both as to the number and as to the shapes of individual chromosomes (figures 7–10). A pair of V-shaped equal armed chromosomes (X^1) appear similar to the X chromosomes, and the three pairs of rods and one pair of very small dots (so small that in some plates they are invisible) seem similar to the autosomes of *Drosophila pseudoobscura*. Females of both species have $2n = 10$. *Drosophila miranda* males (figures 1–6) are, however, unique among the known species of Drosophila in having an odd number of chromosomes ($2n = 9$).

Two pairs of rod-shaped and one pair of dot-shaped chromosomes are similar in males and in females. These are, then, the autosomes of *Droso-*

phila miranda. Instead of the third pair of rods present in the females, the males show a single rod-shaped chromosome (X^2) having no partner. Only one V-shaped equal armed chromosome is present in the male (X^1); its partner is a somewhat shorter, V-shaped, more or less clearly unequal-armed chromosome (Y). The latter chromosome appears similar to the Y chromosomes of some strains of race B of *Drosophila pseudoobscura*



FIGURES 1–10.—Cameralucida drawings of chromosomes of *Drosophila miranda*. FIGURES 1–4.—Spermatogonial metaphase plates. FIGURES 5 and 6.—Nerve cell divisions in the ganglia of male larvae; FIGURES 9 and 10.—Same, in female larvae. FIGURES 7 and 8.—Oogonial divisions. X^1—the X^1 chromosome; Y—the Y chromosome; X^2—the X^2 chromosome having no mate. The scale below represents 10 micra.

(DOBZHANSKY and BOCHE 1933, DOBZHANSKY in press). In the spermatogonia the chromosomes tend to form a definite pattern (figures 1 and 4). The V-shaped chromosomes and the unpaired rod occupy the two ends of a diameter, the rod-shaped autosomes lie between the unpaired rod and the V's, and the dots are at the center.

The only possible interpretation of the chromosome complex of the *miranda* male is that this species possesses two distinct X chromosomes, namely the equal-armed V (to be referred to as the X^1 chromosome), and the rod which has no mate in the male (the X^2 chromosome). The female has, then, $X^1X^1+X^2X^2+$three pairs of autosomes, and male is X^1+X^2+Y +three pairs of autosomes.

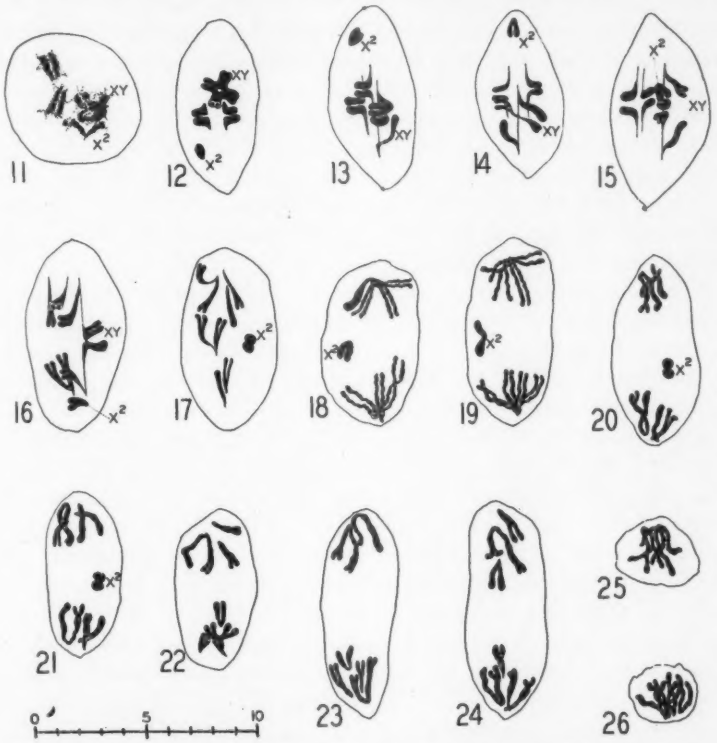
Such a situation seemed so extraordinary in a species of Drosophila that care was taken to discover any possible mistake. In some plates (figures 1, 2, 4) the X^2 chromosome shows the equational split more clearly than the rest of the chromosomes. Is it possible that the X^2 is not really single but represents two separate chromosomes showing an unusually intimate somatic pairing? This question is to be answered in the negative, because: (1) the X^2 in the nerve and the oogonial cells shows no more doubleness than the rest of the chromosomes, (2) in no case does the X^2 chromosome manifest a quadripartite structure, (3) the behavior of X^2 in the spermatogenesis (see below) is incompatible with the above assumption; and finally, (4) the observations on the salivary gland chromosomes afford conclusive proof of the singleness of the X^2. In the salivary glands of the fully grown larvae and young pupae the chromosomes undergo pairing, and the paired homologs appear as cross-striped worm-like bodies. If a chromosome has no homolog in the diploid group, as in the cases of the X chromosomes of *melanogaster*, *simulans*, and *pseudoobscura*, it appears in the salivary gland cells as a body that is paler and narrower than the paired chromosomes. Disregarding the very small dot-like autosomes, the salivary gland cells of *miranda* females contain five darkly staining strands (two limbs of the X^1 chromosome+two autosomes+the X^2 chromosome). In *miranda* males two dark strands (the two autosomes) and three light strands (X^1 and X^2) are visible. In *pseudoobscura* males three dark (three autosomes) and two light strands (two limbs of the X chromosome) are observed.

SPERMATOGENESIS

The above information on the chromosome group of the *Drosophila miranda* male makes an investigation of the spermatogenesis in this species a logical necessity. For a mechanism must be present, whereby a proper distribution of the two X chromosomes (X^1 and X^2) at the reduction division is effected, thus allowing the peculiar method of sex determination observed in this species to perpetuate itself. Testes of young adult males were fixed in Flemming's and Navashin's fluids, and sections (7μ thick) were stained with iron haematoxylin or gentian violet.

Spermatogenesis of *Drosophila pseudoobscura* has been studied by METZ (1926), KOLLER (1933, 1934), DOBZHANSKY (1934) and DARLING-

TON (1934). Spermatogenesis in *miranda* is in general similar to that in *pseudoobscura*, thus permitting us to make a comparative description. The general structure of the testis, of the spermatogonia, and of the first spermatocytes is similar. The stainability of chromosomes in prophase stages of meiosis is even poorer in *miranda* than in *pseudoobscura*. At stages up to and including diakinesis the chromosomes appear as more or



FIGURES 11–26.—Diakinesis and the first meiotic division in *Drosophila miranda*. XY—the X^1Y bivalent; X^2—the X^2 chromosome (the chromosomes are marked only in the figures in which they can be identified with certainty). The scale below represents 10 micra.

less pale brown clumps. At late diakinesis (figure 11) four bodies can be seen in the nucleus; one of them, the largest, is probably the bivalent composed of the X^1 and the Y chromosomes; two others are the autosomal tetrads, and the fifth is the X^2 chromosome. The X^2 does not seem to occupy any definite position with respect to the X^1Y bivalent.

The first meiotic division (figures 12–24) is frequently very clear in *miranda*. The chromosomes are somewhat longer and more slender than

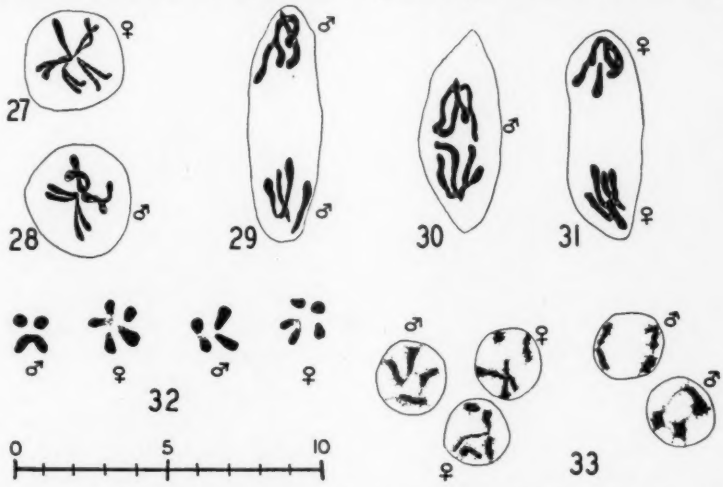
in *pseudoobscura*. At metaphase and early anaphase the X^2 may lie anywhere on the spindle, in the equatorial region (figure 15) or near one of the poles (figures 12–14, 16). This chromosome is very much condensed, the equational split shows clearly in most cases (figures 13–16), and its spindle fibre attachment is directed toward (figure 14) or away from (figures 13, 16) the nearest pole. It is to be emphasized, that there is no apparent physical connection between the X^2 chromosome and the X^1Y bivalent, nor does it occupy any definite position with respect to the latter. The two diads composing an autosomal bivalent are frequently separated from each other by a much wider space than is the case in *pseudoobscura* (see especially figure 15). This corroborates the observations of DARLINGTON (1934) and DOBZHANSKY (1934) according to which no chiasmata are formed in the autosomal bivalents in Drosophila males. On the other hand, the X^1Y bivalent assumes shapes that were interpreted by DARLINGTON (l.c.) as indicating the presence of two chiasmata in this bivalent (figure 12, also figures 13–16).

At late anaphase and early telophase the autosomes and the X^1 and Y may be seen lying at the poles of the spindle (figures 16–21). Their bodies are long and slender, and the equational split is frequently very clearly visible (figures 18 and 19; it is not certain whether figures 18 and 19 represent an earlier stage than figures 20 and 21, or *vice versa*). The X^2 comes to lie in the equatorial region of the spindle, its body having the shape of a dumb-bell or of a very short and stout V, and its spindle fibre attachment still showing no definite orientation with respect to the poles (figures 17–21). At the next stage (figures 22–26) the X^2 chromosome is included in one of the telophasic groups, its spindle attachment now clearly pointed toward the nearest pole, and its shape no longer different from that of the rest of the chromosomes.

The first meiotic division is, consequently, reductional both for the X^2 chromosome and, probably, for the X^1Y bivalent. It is frequently possible to count the chromosomes in both telophasic groups in the same cell, and to ascertain that one of these groups may contain four and the other three chromosomes, a V-shaped chromosome being present in each. Since, however, the X^1 and Y chromosomes are not distinguishable at this stage, it is impossible to determine whether the X^2 goes to the same pole with the X^1 or with the Y chromosome.

In contradistinction to *Drosophila pseudoobscura*, the second spermatocytes (figures 25, 26) in *Drosophila miranda* seem to exist only for a very short time. No resting stage intervenes between the first and the second meiotic divisions, and the chromosomes pass onto the spindle of the second division without disentangling themselves from the telophasic snarls. Because of this, the metaphases of the second divisions are not clear in

miranda, and counting the chromosomes is possible only in exceptional cells (figures 27, 28). The anaphases and telophases are again clear enough (figures 29–31), and it is easy to see that two kinds of cells are present. In some cells (figures 28-30) three chromosomes, including one V-shaped one, migrate to each pole, while in other cells (figures 27, 31) four chromosomes are involved. Evidently, the former kind is devoid of X^2 chromosome and is destined to produce male-determining sperms, and the latter kind contains the X^2 chromosome and gives female-determining sperms. Counting chromosomes is especially easy in telophasic groups observed in polar view (figure 32). In 102 groups so counted, 53 contained three chromosomes and 49 four chromosomes, a close approximation to a 1 : 1 ratio.



FIGURES 27–33.—The second meiotic division (figures 27–32) and the nuclei of young spermatids (figure 33) in *Drosophila miranda*. Cells giving rise to female- and male-determining spermatozoa are marked ♀ and ♂ respectively. The scale below represents 10 micra. All drawings are made with the aid of a camera-lucida.

The spermatids and the spermiogenesis are apparently alike in *miranda* and *pseudoobscura*, with the distinction that in the former the chromosomes in the spermatids do not fuse into a single body immediately after the second division as they do in the latter. The nuclei of the young spermatids (figure 33) frequently show separate and countable chromosomes; some nuclei contain three, and others four chromosomes. The bundles of spermatozoa consist of a number of threads approaching 128, the same number as observed in *pseudoobscura*. No spermatids or spermatozoa are visibly abnormal.

The result arrived at is an anomalous one. At no stage of meiosis is the

X^2 chromosome paired with either X^1 or Y, and at the first division the X^2 proceeds to one of the poles seemingly undirected by the distribution of the X^1 and Y. An independent distribution of the X^2 with respect to the X^1 and Y must, however, give rise to four kinds of spermatozoa: (1) —X^1X^2, (2)—Y, (3)—X^1, (4)—X^2Y. Since the female is the homozygous sex, all eggs must be X^1X^2. Accordingly, four classes of zygotes should be produced: (a)—$X^1X^1X^2X^2$, (b)—X^1YX^2, (c)—$X^1YX^2X^2$, and (d)—$X^1X^1X^2$. Among the adult individuals we find only classes (a) and (b). Hence, gametes (3) and (4) are either not formed at all, or give rise to inviable eggs, or some chromosomes are eliminated in the eggs after fertilization, perhaps in a manner similar to that described by METZ (METZ, MOSES and HOPPE (1926), METZ and SCHMUCK (1931)) in Sciara. To discriminate between these possibilities, observations on the sex ratio and the viability of the eggs were made.

THE SEX RATIO AND THE VIABILITY OF THE EGGS

Sex of the offspring from pair matings is recorded in table 3. No unisexual progenies are produced. The sex ratio in the total is 100 ♀ ♀ to 75.3 ♂ ♂. The deficiency of males is probably due to their slow developmental rate (see above) which puts them at a disadvantage in the cultures. It may be noticed that cultures producing few offspring give a sex ratio approaching unity.

TABLE 3

Sex ratio in Drosophila miranda.

CULTURE NO.	♀♀	♂♂	CULTURE NO.	♀♀	♂♂
1	56	45	7	43	29
2	65	40	8	45	33
3	15	16	9	100	56
4	95	66	10	68	63
5	90	81			
6	17	18	Total	594	447

Drosophila miranda females were allowed to deposit eggs on paper spoons with food and a small amount of yeast. The number of larvae produced and the number of eggs that failed to hatch were recorded. The result is shown in table 4.

In another experiment (table 5) the spoons with a known number of eggs were placed in regular culture bottles, and the adults obtained were counted.

The mortality in the offspring of *Drosophila miranda* is obviously insufficient to account for the elimination of the 50 per cent of male gametes and the resulting zygotes that may theoretically be produced. In fact, the ob-

TABLE 4

Viability of the eggs.

SPOON NO.	EGGS LAID	LARVAE HATCHED	PERCENT OF HATCHING	SPOON NO.	EGGS LAID	LARVAE HATCHED	PERCENT OF HATCHING
1	16	12	75	6	58	52	89½
2	96	92	96	7	47	45	96
3	18	18	100	8	47	47	100
4	92	86	93½	9	36	36	100
5	90	83	92				
				Total	500	471	94.2

TABLE 5

Number of adults derived from a given number of eggs.

CULTURE NO.	NUMBER OF EGGS	♀♀	♂♂	TOTAL	PERCENT OF SURVIVAL
1	62	30	22	52	84
2	19	8	8	16	84
3	13	9	4	13	100
4	29	14	12	26	89½
5	33	16	10	26	79
6	26	13	9	22	84½
7	27	10	8	18	66½
8	13	6	4	10	77
Total	222	106	77	183	82.4

served morality is only slightly greater than necessary to account for the deviation from the 1:1 sex ratio among the adults.

The conclusion is forced on us that the two classes of gametes not represented among the adults are either not produced at all, or are somehow eliminated before fertilization. Since there is no indication that the latter possibility is realized, it seems probable that only X^1X^2 and Y gametes are produced in spermatogenesis. To put it another way, the disjunction of X^2 is not independent of that of the X^1Y bivalent, but on the contrary, the X^2 chromosome always passes to the same pole of the spindle of the first meiotic division as does the X^1 chromosome. The *modus operandi* of the mechanism by which such a determinate disjunction is brought about is, however, not at all clear.

HYBRIDS BETWEEN DROSOPHILA MIRANDA AND DROSOPHILA PSEUDOOBSCURA

A preliminary account of the results of hybridization of the two species under consideration may be given here, a fuller account being reserved for a separate publication. There is no great difficulty in crossing *Drosophila miranda*, either as a female or as a male, either to A or to B race of *Droso-*

phila pseudoobscura. The results of crosses to A and B races are apparently similar. The cross *miranda* ♀ × *pseudoobscura* ♂ produces adult offspring of both sexes in approximately equal numbers. Hybrid females are normal morphologically, slightly closer in size to *miranda* than to the other parent, and, after a rather long delay in the maturation of the ovaries, produce numerous eggs none of which however hatches. The eggs are normal externally. Hybrid males hatch late, and are visibly abnormal—short, squat, somewhat flattened bodies, rough eyes, spread wings, misshapen legs. Their testes are very small and contain nothing resembling mature sperm. The cross *pseudoobscura* ♀ × *miranda* ♂ produces mostly females (about 1 ♂ ♂ : 1,000 ♀ ♀). Hybrid females are similar to those from the reciprocal cross. The occasional males are less abnormal morphologically than hybrid males from the reciprocal cross, but still have very small testes and are completely sterile.

DISCUSSION

The morphological differences between *Drosophila miranda* and *pseudoobscura* are relatively slight, and subject to a rather considerable variation due to environmental influences. The result is that classifying a single individual developed under unknown external conditions as belonging to one or the other species may be hazardous. To be certain, if the individual in question is a living male, its species can be securely established by an examination of its chromosomes. A taxonomist accustomed to determining species of dead and dried specimens will, however, derive little comfort from this consideration, and few geneticists will seriously argue that he should change his habits in this respect. Is, then, the elevation of *Drosophila miranda* to specific rank justified? In the opinion of the writer this question should be answered in the affirmative.

The category of the species is basically different from all other taxonomic categories. The latter are "natural" only insofar as they reflect the discontinuities actually existing in the living world at a given time level, but the general scheme of classification is determined essentially by its expediency. Species possesses all the phenomenological and all the pragmatic attributes of the rest of the taxonomic categories, plus an attribute peculiar only to itself. A species is a system the members of which, because of their physiological properties, do not interbreed with members of other similar systems. (Races that are prevented from interbreeding by geographic isolation, but which are fully inter-fertile when brought together by natural or artificial means, are, thus, not to be considered distinct species.) Considered dynamically, the species represents that stage of evolutionary divergence, at which the once actually or potentially interbreeding array of forms is segregated into two, or more, separate arrays

that are physiologically incapable of interbreeding. It would be out of place here to discuss in detail how widely the above criterion can be consistently applied. Its application is admittedly not universal; for example, it is restricted to habitually cross-fertilizing organisms. It is, however, apparent that *Drosophila miranda* and *pseudoobscura* must be considered specifically distinct, since they are isolated by the complete sterility of their hybrids. Non-transgressive morphological differences are not a necessary prerequisite of the specific distinction, since the genetic factors preventing interbreeding (that is, factors determining the inviability of the hybrid offspring, or the hybrid sterility, or the sexual isolation may or may not be reflected in morphological structures. Closely related species of Drosophila (for example, *melanogaster* and *simulans*) frequently show a paucity of morphological differentials.

The nature of the specific difference between *miranda* and *pseudoobscura* constitutes an interesting problem. A cycle of physiological differences makes *miranda* an ecological type separate from *pseudoobscura*. Spermatogenesis in *miranda* deviates from that in *pseudoobscura* not only in those features that are clearly related to the presence of an unpaired chromosome in *miranda*, but also in a number of other respects (the failure of the meiotic prophase chromosomes to stain, the elimination of the interphase between the two meiotic divisions, the visibility of chromosomes in the spermatids). The most striking peculiarity of *miranda* is the X^1-X^2-Y mechanism of sex determination. *Miranda* has one pair of autosomes less, and one sex chromosome more, than *pseudoobscura*. It is very unlikely that the X^2 chromosome of *miranda* is completely homologous to one of the autosomes of *pseudoobscura*. The superficial similarity of the chromosomes in the females of both species is undoubtedly deceiving, concealing, as it does, a far reaching remodeling of the chromosome apparatus. A preliminary study of the salivary gland chromosomes in the hybrid larvae, performed by Mr. C. C. TAN and the writer, fully corroborates this conclusion. (Note added in proof: it has been found that not a single chromosome of *miranda* is identical with any chromosome of *pseudoobscura;* even the small dot-like chromosomes are different. Besides numerous inverted sections, frequently very small in extent and covering only a few bands, a number of translocations between the chromosomes are observed. The, X^2 chromosome of *miranda* contains at least one section that in *pseudoobscura* is located in the X chromosome, and the fourth chromosome of *pseudoobscura* includes at least one section whose homolog is found in the X^1 chromosome of *miranda*.)

The origin of *pseudoobscura* and *miranda* from their hypothetical common ancestor must have involved a series of elaborate changes in the mechanisms whose precise functioning is essential if the race is to endure.

Not only has the arrangement of the genic material in the chromosomes been altered, but a mechanism of determinate disjunction of heterochromosomes at the reduction division has been evolved. In fact, such a mechanism is, to the writer's knowledge, not found in any other form with the possible exception of the insufficiently established case of a race of *Gryllotalpa vulgaris* described by VOINOV (1914). Multiple X's and Y chromosomes are, of course, not infrequent in various groups of animals and also in plants (a review in SCHRADER 1928), but at least in most of such cases the multiple heterochromosomes pair with each other at meiosis, and this may be presumed to insure their regular disjunction. The spermatogenesis of Sciara (METZ 1929, 1933) constitutes the only real, though very remote, parallel to that in *Drosophila miranda*.

SUMMARY

1. *Drosophila miranda* occurs in the state of Washington and in the adjacent part of British Columbia.

2. *Drosophila miranda* differs from *Drosophila pseudoobscura*, a species to which it is very closely related, by a somewhat larger body size, larger sex combs in the male, longer period of development, and a greater sensitivity to heat. The classification of dead specimens is hazardous.

3. The male of *Drosophila miranda* has an odd number of chromosomes ($2n=9$), among which one heteromorphic pair (the X^1 and Y chromosomes), and one unpaired chromosome (X^2) are present. The female has ten chromosomes and no heteromorphic pairs.

4. This condition is interpreted as meaning that the species has two distinct X chromosomes, X^1 and X^2. The male is X^1YX^2, and the female $X^1X^1X^2X^2$.

5. The X^1 and Y chromosomes form a bivalent at meiosis. The X^2 chromosome remains unpaired. During the first meiotic division the X^2 lags on the spindle, and finally passes undivided in one of the telophasic groups. The X^2 divides normally at the second division.

6. Evidence is presented to show that the X^2 passes at the first division to the same pole with the X^1 chromosome. Two, instead of the possible four, types of male gametes are formed. The nature of the mechanism insuring this determinate disjunction is unknown.

7. Spermatogenesis in *Drosophila miranda* differs from that in *pseudoobscura* in some further particulars not clearly related to the heterochromosome situation. Thus, in *miranda*, the interphase between the first and the second divisions is practically eliminated, and the chromosomes remain for a certain time visible in the spermatids.

8. *Drosophila miranda* can be crossed to *pseudoobscura*. The cross *miranda* ♀ × *pseudoobscura* ♂ produces offspring of both sexes, while the re-

ciprocal cross produces predominantly females, and only occasionally a few males. The hybrids of both sexes are completely sterile. In addition, the male hybrids are somatically abnormal.

9. The nature of the specific difference between *miranda* and *pseudo-obscura* is discussed.

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SALIVARY GLAND CHROMOSOMES IN THE TWO RACES OF DROSOPHILA PSEUDOOBSCURA

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Received February 13, 1935

INTRODUCTION

The two races of *Drosophila pseudoobscura*, known as race A and race B, produce sterile male offspring when crossed (LANCEFIELD 1929), have visibly different shapes of the Y chromosomes (DOBZHANSKY and BOCHE 1933), and differ in their physiological characteristics (POULSON 1934, DOBZHANSKY 1935a, BOCHE unpublished). They appear, however, indistinguishable morphologically. LANCEFIELD (1929) and KOLLER (1932) found, in female hybrids between these two races, a suppression of crossing over as observed in both ends of the X chromosome, while in its middle crossing over is nearly normal. Such a suppression of crossing over is usually due to the presence of inverted sections (STURTEVANT 1926, 1931). It is, therefore, probable that race A differs from race B in having two inverted sections in the X chromosome. Nothing has been reported concerning the conditions of autosomes in this respect.

The technique of studying the salivary gland chromosomes (HEITZ 1933 and PAINTER 1934a) permits a more precise comparison of the gene arrangement in the chromosomes of related species and races of Diptera than has been possible heretofore. The results of such a comparison of the chromosomes of A and B races of *Drosophila pseudoobscura* are presented in this paper. The author wishes to express his gratitude to Professors TH. DOBZHANSKY and A. H. STURTEVANT for their encouragement, advice and use of their stocks

MATERIAL AND METHODS

Strains of *Drosophila pseudoobscura* coming from different parts of the distribution area of this species were used. A collection of such strains is being kept in this laboratory. The aceto-carmine smear method for studying the salivary gland chromosomes has been applied as described by PAINTER (1934a and b). Larvae whose chromosomes were to be studied were raised at low temperature (14°C). It has been noticed by the writer, and recently also by HEITZ (1934), that chromosomes in such larvae are larger, thicker and can be better stretched in preparations than in larvae grown at higher temperatures. The slides were preserved at the same low temperature, which keeps them longer in a good condition for observation.

All drawings were made with the aid of a camera lucida. Figures 1 to 6 are really composite drawings. Each section of the chromosomes has

been copied from a camera lucida sketch showing this particular section most clearly. The banding in each section has been checked at least twice in different preparations. It is not claimed that these figures show the full number of bands present in the salivary gland chromosomes of the species, but it is hoped that they are good enough for a demonstration of at least qualitative differences in the gene arrangement in the chromosomes and as a basis for future studies.

GENERAL CHROMOSOME MORPHOLOGY

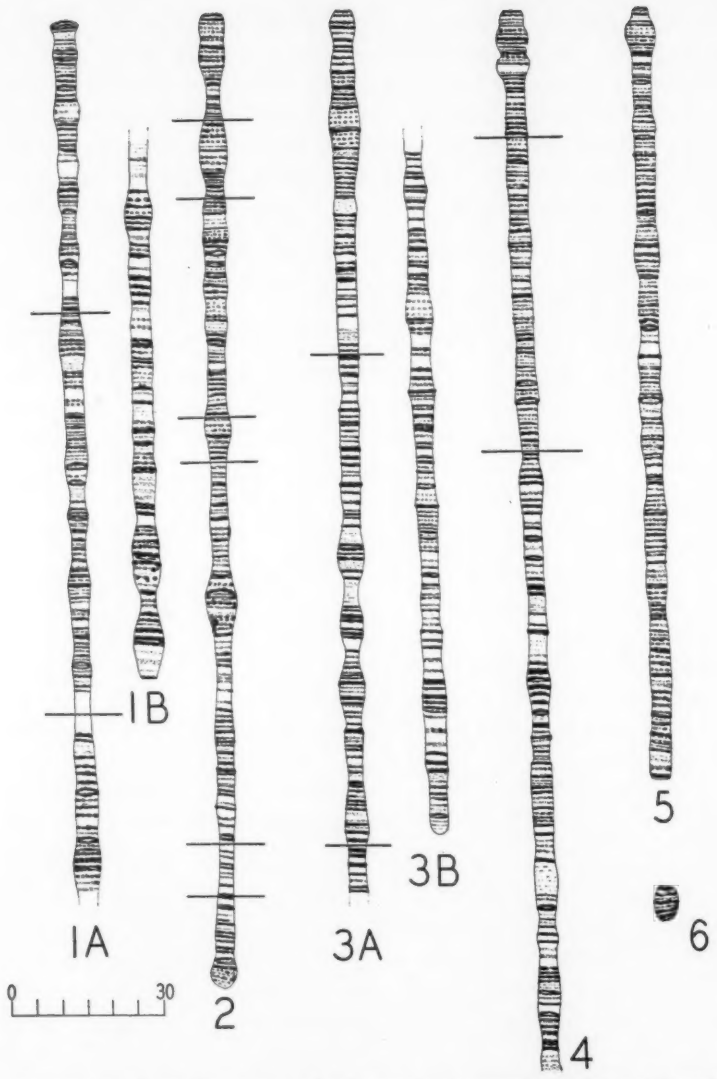
The chromosome group of *Drosophila pseudoobscura*, as seen in the spermatogonial, oogonial or nerve cell division, consists of five pairs of chromosomes: V-shaped X chromosome, three rod-shaped autosomes, and one very small dot-like autosome. In the salivary glands of adult larvae or young pupae five long strands, each embedded at one of the ends in a chromatin coagulum known as the chromocenter can be seen (figure 10). In favorable cases, a very short (sixth) element may be seen attached to the chromocenter. This is supposedly the dot-shaped autosome (figure 6). Each strand represents two closely paired homologous chromosomes, the result of somatic synapsis (HEITZ 1933).

In females all the strands are equally densely stained and similar in width. In males two of the long strands are distinctly paler and somewhat thinner than the rest. The two paler strands are the two limbs of the X chromosome. The Y chromosome, being apparently composed of an inert material, is not paired with the X, but is probably included in the chromocenter, just as it is in *Drosophila melanogaster* (PAINTER 1934b, HEITZ 1934).

Individual chromosomes can be identified in any nucleus, once one is acquainted with their distinctive characteristics, especially in the regions of their free ends. The correspondence between the respective chromosomes in the salivary gland cells and their counterparts among the linkage groups known in the species has been determined by studying the inverted sections in the different chromosomes. The autosomes were studied mostly in the preparations of male larvae, since in these the autosomes stand out distinctly from the lighter and more slender X chromosome. Figures 1 to 6 show the composite maps of the chromosomes of the race A.

THE X CHROMOSOME

The X chromosome (figures 1 and 2) is recognized by being thicker and more intensely stained in females than in males (see above). The two arms are of unequal length in salivary gland cells, one arm (figure 2) being about two-thirds of the length of the other (figure 1). Since in cells other than those of the salivary glands, the X chromosome is equal-armed



FIGURES 1–6.—Composite drawings of the salivary gland chromosomes in race A of *Drosophila pseudoobscura*. Figure 1—the right limb of the X chromosome (the figure cut in two; 1A—the distal part, 1B—the proximal part). Figure 2—the left limb of the X chromosome. Figure 3—the second chromosome (3A—distal, 3B—proximal part). Figure 4—the third chromosome. Figure 5—the fourth chromosome. Figure 6—the fifth chromosome. The distal ends are directed upwards in all figures. The approximate lengths of the inversions distinguishing races are shown by cross bars. The left limb of the X chromosome has three inversions, the distal one being relatively somewhat longer than suggested by the drawing. The scale below represents 30 micra.

(DOBZHANSKY and BOCHE 1933), the behavior in salivary gland cells suggests that one of the arms contains more inert material than the other. In most salivary gland preparations, there is no indication that the two arms of the X chromosome belong together, but occasionally, on crushing the nucleus, they are separated from the rest of the chromosomes together with a part of the chromocenter adjoining them.

Race A females carrying the dominant sex-linked gene Pointed have been repeatedly crossed to B race males. After many such crosses flies were obtained that carried Pointed and a section of the X chromosome adjacent thereto from race A, and had all other chromosomes replaced by those of race B. Linkage tests show that females carrying this X and a race B X give normal crossing over in the right end of X, and greatly reduced crossing over in the left end. The salivary gland cells of such females heterozygous for Pointed were studied (the flies were supplied by Dr. A. H. STURTEVANT). A long and two very short inverted sections have been found in one of the limbs of the X chromosome (figure 11). Since the locus of Pointed lies at the extreme left end of the known map of the X chromosome, the limb showing these inversions must be the left limb of the X. This limb appears, in the salivary gland cells, shorter than the other limb, which, consequently, must be the right limb of the X chromosome.

Race A females homozygous for the sex-linked recessive genes beaded, yellow and short were crossed to race B wild-type males. The F_1 females were backcrossed to beaded yellow short males, and in the offspring some beaded non-yellow non-short females were selected. Since beaded lies near the left end (not very far from Pointed), yellow about in the middle, and short at the right end of the known map of the X chromosome, these females presumably had the right limb of one of their X chromosoms from race B, and the rest of the X chromosomes from race A. A part of the offspring of such females (crossed to race A males) showed an inverted section in the longer limb of the X chromosome (figure 12). The longer limb of the X chromosome in the salivary gland cells is, accordingly, the right limb.

Besides the difference in length, the two limbs of the X chromosome can be distinguished in salivary gland preparations by the characteristics of their free end portions. The free end of the right limb (figure 1) is inflated to form a disc-like structure. Just beyond the disc there is a light area, followed by about twelve heavy and light bands lying rather close together. Next follow an inflated lightly staining region, and a group of about six or seven heavy bands and a second inflated region and a constriction which usually makes a good landmark. The left limb (figure 2) terminates in an oblong, square-headed bulb, showing a number of dis-

tinct bands and vesiculated areas, among which one band, lying at about the middle of the bulb, is particularly heavy. Proximally the bulb is separated from the body of the chromosome by a neck-like constriction, the distal end of which is also marked by two heavy bands.

THE SECOND CHROMOSOME

The second chromosome (figure 3) is the carrier of the Bare-Smoky linkage group. In the salivary gland cells the second chromosome is the longest in the nucleus (considering each limb of the X as a separate chromosome). It has been identified by means of an inversion, present in the

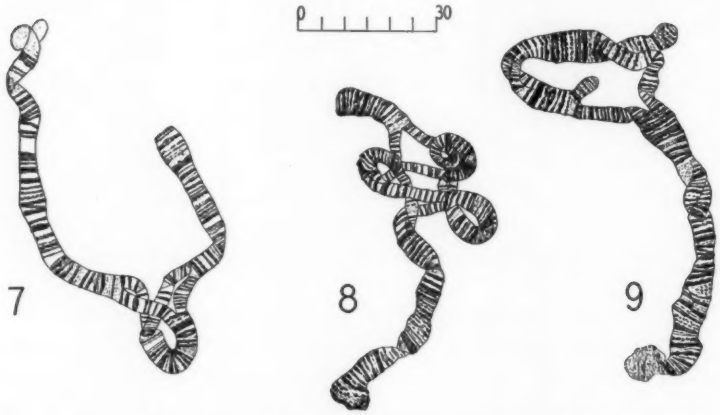


FIGURE 7.—Inversion figure in third chromosome in the hybrid San Bernard ino-1 ♀ × *or Sc pr* ♂.

FIGURE 8.—A complex configuration in the third chromosome given by two overlapping inversions (A cross San Bernardino-1 ♀ × Seattle-4 ♂).

FIGURE 9.—An inversion figure in the second chromosome (A cross Santa Lucia-7 ♀ × Bare-Smoky ♂). The scale above represents 30 micra.

strain Santa Lucia-7, which has been found by Dr. STURTEVANT to reduce very markedly the crossing over between the genes Bare and Smoky. One half of the larvae from the cross (Santa Lucia-7 females × Bare-Smoky males) F_1 females × cinnabar males were found to possess in their salivary gland cells the inversion figure, an example of which is shown in figure 9.

Cytologically, the second chromosome resembles the right limb of the X chromosome. Both have terminal bulbs carrying rather heavy bands. However, in the second chromosome there exists a large vesiculated area not far from the free end; this area carries a heavy band in its middle, and is preceded by four very heavy bands. Distally, the free end is more or less club-shaped rather than disc-shaped as in the X chromosome.

THE THIRD CHROMOSOME

The third chromosome (figure 4) carries the orange-Scute-purple linkage group (CREW and LAMY 1934). STURTEVANT (unpublished) observed that a large number of wild strains have an inverted section in this chromosome (as judged by a reduction of crossing over in the hybrids between these strains and *or Sc pr*). Two of the strains in which such a reduction of crossing over has been observed, namely, Santa Lucia-37 and San Bernardino-1, were crossed to *or Sc pr*. The salivary glands of the F_1 larvae were examined, and inversion figures, an example of which is shown in figure 7, were found in every cell.

The free end of the third chromosome resembles somewhat that of the left limb of the X. However, in the third chromosome there exists a rather prominent inflated region very close to the free end instead of the constriction observed in the left limb of the X in approximately the same position. Distally from this inflated area, which is devoid of prominent bands, is located a knob-like end portion with heavy bands.

THE FOURTH AND FIFTH CHROMOSOMES

The two remaining autosomes (figures 5 and 6) are extremely unlike in size. The fifth obviously corresponds to the dot-like chromosome in the metaphase plate. The correspondence between these chromosomes and the linkage groups is a matter of conjecture since no inversions have been as yet discovered in either.

The fourth chromosome (figure 5) is shorter than any other chromosome in the salivary gland cells except the fifth, which is very small. The fourth is easily distinguished from the rest by a conspicuous terminal inflation. The fifth (figure 6) is frequently invisible due to its smallness and to being imbedded in the chromocenter. It is elliptical in shape, and shows five heavy and several light bands.

CHROMOSOME DIFFERENCES BETWEEN RACE A AND RACE B

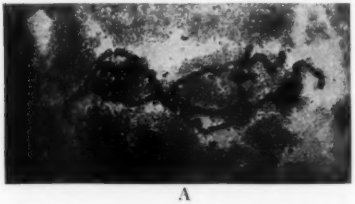
Salivary gland cells of hybrid larvae from the crosses of race A and race B show one large and two very small inverted sections in the left limb of the X chromosome (figure 11), and rather long inversions in the right limb of the X chromosome, in the second and in the third chromosomes (figures 12, 13 and 14). In figure 10 all six inversions can be seen.

These inversions have been observed in the following interracial crosses:

Texas(A) ♀ × Seattle-4(B) ♂	*or Sc pr* (A) ♀ × Cape Flattery-3(B) ♂
Texas(A) ♀ × Seattle-6(B) ♂	*or Sc pr* (A) ♀ × Campbell Riv.-3(B) ♂
La Grande-2(A) ♀ × Seattle-4(B) ♂	*or Sc pr* (A) ♀ × Quesnel-5(B) ♂
La Grande-2(A) ♀ × Seattle-6(B) ♂	*or Sc pr* (A) ♀ × Greenhorn Mt.-3(B) ♂
La Grande-2(A) ♀ × La Grande-4(B) ♂	*or Sc pr* (A) ♀ × Lassen-7(B) ♂

Zion-1(A) ♀ ×Seattle-4(B)♂ *or Sc pr*(A) ♀ ×Humboldt-1(B)♂
San Bernardino-1(A) ♀ ×Seattle-4(B)♂ *or Sc pr*(A) ♀ ×Reedsport-3(B)♂
Lassen-16(A) ♀ ×Seattle-4(B)♂ *or Sc pr*(A) ♀ ×La Grande-4(B)♂
Aspen(A) ♀ ×Seattle-4(B)♂ *or Sc pr*(A) ♀ ×Seattle-4(B)♂
Idaho-Falls(A) ♀ ×Seattle-4(B)♂ Shuswap-1(A) ♀ ×Seattle-4(B)♂
bd y sh(A) ♀ ×Seattle-4(B)♂

Since inverted sections are by no means infrequent in different chromosomes in various strains collected in nature (unpublished data of Dr. STURTEVANT, see above), it was necessary to establish whether these inversions observed in the interracial hybrids are racial characters or char-



A B

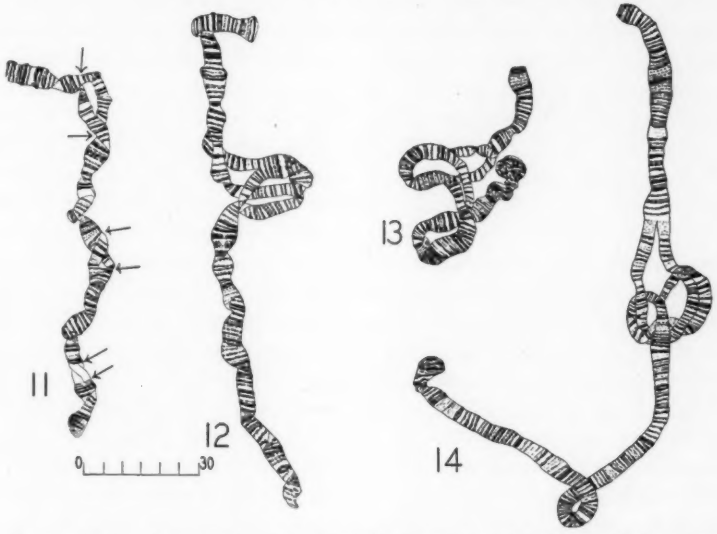
FIGURE 10.—A—a microphotograph of the salivary chromosome complement of A×B hybrid showing all interracial inversions. B—an outline sketch of the figure on the left with each chromosome and inversion labelled.

acters of individual strains. In the crosses listed above both A race and B race strains were used coming from diverse parts of the distribution area of each race. Likewise, strains of race A and race B of different "strength" and having different types of Y chromosomes (DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1935b) were used. Since in all crosses with the apparent exception of the cross or *Sc pr* ♀ ×Greenhorn Mt.-3♂, all the inversions mentioned above were cytologically identified, these inversions may be safely considered to constitute distinctive properties of races. In the exceptional cross the third chromosome of the hybrid had no inverted section.

The three inversions observed in the hybrid in the left limb of the X chromosome are shown in figure 11. One of these inversions, located most distally, is the longest in this chromosome. The other two, one at the middle and the other in the proximal portion of the chromosome, are exceedingly short. Each of these, especially the proximal one, includes very few bands. In view of this the identification of these two inversions is based on the consistent lack of pairing in those regions rather than on a definite alteration of the sequence of the bands (these regions pair normally in pure races). However, the possibility is not excluded that these are not inversions at all. The inverted section in the right limb of the X, in the second and in the third chromosomes are shown respectively in figures 12, 13 and 14. All of these involve relatively long sections of chro-

mosomes (their limits are marked in the composite chromosome maps in figures 1, 2, 3 and 4).

None of the inverted sections found within a race (see above) seem to be identical with the inversions distinguishing races. As stated above, a number of A race strains found in nature have an order of genes different from that characteristic for the *or Sc pr* strain of the race A, and show inversion figure when crossed to the latter. One of these strains, namely San Bernardino-1, has been crossed to race B (Seattle-4). In the hybrid the third chromosome showed a peculiar configuration (figure 8) which



FIGURES 11–14.—Inversions in the A×B hybrids. Figure 11—left limb of the X chromosome; Figure 12—right limb of the X; Figure 13—the third chromosome; Figure 14—the second chromosome. Arrows in figure 11 point at the inversions. The scale below represents 30 micra.

can be accounted for on the assumption that this hybrid had two different inversions in this chromosome. The non-identity of other interracial inversions with the intraracial ones has been established by observing that they involve different regions.

Some interesting observations on the pairing of inverted chromosome segments in the salivary gland preparations were made. A complete pairing of all sections of chromosomes is, on the whole, infrequent in such preparations. Very short inversions, such as the two smaller ones in the left limb of X, apparently never pair, although the non-inverted parts adjacent to the inversion usually show complete pairing. In somewhat longer inversions, such as the longest inversion in the left limb of the X

chromosome, pairing of the inverted parts is only seldom observed. Finally in cases of long inversions, such as both interracial and intraracial inversions in the second and third chromosomes and in the right limb of the X, the inverted segments are apparently always paired at least in a part of their lengths, and occasionally are paired completely. On the other hand, in these long inversions the non-inverted parts of the chromosomes adjacent to the inversions almost invariably fail to pair for a certain distance.

DISCUSSION

Comparisons of chromosome groups in closely related species frequently show dissimilarities both in the number and in the morphology of chromosomes. It seems probable that these cytologically observable dissimilarities are merely visible manifestations of the differences in the internal structure of the chromosomes, or more precisely, in the gene arrangement. STURTEVANT (1921, 1929) was the first to show, in the case of *Drosophila melanogaster* and *Drosophila simulans*, that such differences between species really exist. The third chromosome of *Drosophila simulans* has an inverted section as compared with *Drosophila melanogaster*.

As shown by the sterility of their hybrids, race A and race B of *Drosophila pseudoobscura* may be regarded an incipient species. The data presented above show that rearrangements of the genic material within the chromosome took place during the processes that led to the separation of these two races. Six inverted sections, four in the X chromosome, and one in each of the second and the third chromosomes, are present in the interracial hybrids. Since a rather large number of strains coming from different parts of the distribution area were involved in the hybrids in which the inversions were discovered, it is reasonable to assume that these inversions are racial characters.

Whether the inverted sections distinguishing the races are directly responsible for the sterility of the interracial hybrids is at least doubtful. DOBZHANSKY 1933a, 1934b) adduced a number of arguments to prove that the sterility of the *Drosophila pseudoobscura* hybrids is due to an interaction of complementary factors rather than to differences in the gross structure of the chromosomes. Moreover, judging by analogy with *Drosophila melanogaster*, an individual heterozygous for several inverted sections in different chromosomes need not be sterile. There still, however, remains a possibility that sterility is somehow indirectly connected with these intraracial inversions. The gene mutations that cause sterility might have been in some manner connected in their origins with the origin of the inversions, just as some mutations, or position effects, are connected with translocations and inversions.

Whether or not the variations in the gene arrangement are causally

related to the hybrid sterility in *Drosophila pseudoobscura*, it remains a fact that racial differentiation partook in this case of both sources of evolutionary variability—gene variability and variability in the gross structure of chromosomes. It is, indeed, probable that the hybrid sterility may be in some cases directly caused by structural differences in the chromosomes of the parental species, especially in the hybrids that produce fertile allotetraploids.

The pairing in the salivary gland cells of the homologous strands carrying inversions deserves some consideration. In case of relatively long inversions, the parts of the chromosome adjacent to the loci of breakage mostly fail to pair with their homologs, while the inverted sections usually pair along most of their lengths, and form rings or similar configurations. On the other hand, short inverted sections frequently fail to pair completely, while the non-inverted parts are regularly paired. These facts are especially interesting if the meiotic pairing in the female germ cells is really analogous with the somatic synapsis in the salivary gland cells. For in this case, the disturbances of crossing over produced by the inverted sections may find their causal explanation, as suggested by the hypotheses of competitive pairing (DOBZHANSKY 1933b, 1934a). Indeed, similar phenomena have been observed by MCCLINTOCK (1933) in the meiosis of individuals of *Zea mays* carrying inversions.

SUMMARY

1. Five long chromosome strands, each attached at one of its ends to the chromocenter, and one small chromosome imbedded in the chromocenter, are visible in the nuclei of the salivary gland cells of the adult larvae of *Drosophila pseudoobscura*.

2. Individual chromosome strands can be identified by their morphological peculiarities—distribution of the "bands," inflated areas, and the shape of the free ends. In the salivary gland cells of the adult larvae, the Y chromosome is invisible, and, in the males, the X chromosome is paler than the autosomes, due to being single instead of composed from two paired homologs.

3. The correspondence between the chromosome strands observed in the salivary gland cells and the linkage groups of genes known in *Drosophila pseudoobscura* has been established by observing individuals carrying inverted sections in one or other of the chromosomes.

4. The two limbs of the X chromosome appear similar in length in the spermatogonial or nerve cell metaphases, but they are unequally long in the salivary gland cells. This is probably due to one of the limbs containing more inert material than the other limb.

5. The two races of *Drosophila pseudoobscura* differ in six inverted sec-

tions: four in the X chromosome, one in the second, and one in the third chromosome.

6. Inverted sections within a race have also been demonstrated cytologically. These inversions occur in nature.

7. Apparently none of the intraracial inversions are identical with the interracial ones.

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